Microchemical Journal 156 (2020) 104854

Contents lists available at ScienceDirect

Microchemical Journal

journal homepage: www.elsevier.com/locate/microc

The determination of fatty acids in cheeses of variable composition (cow, ewe's, and goat) by means of near infrared spectroscopy

M. Inmaculada González-Martín[a], Ana M. Vivar-Quintana[b,*], Isabel Revilla[b], Javier Salvador-Esteban[a]

[a] Department of Analytical Chemistry, Nutrition, and Bromatology. Faculty of Chemical Science, University of Salamanca, Plaza de los Caídos, 37008 Salamanca, Spain
[b] Food Technology Area, Higher Polytechnic School of Zamora, University of Salamanca, Avenida Requejo 33, 49022 Zamora, Spain

ARTICLE INFO

Keywords:
Near infrared spectroscopy (NIR)
Cheese
Fatty acids

ABSTRACT

The fat composition is one of the factors which has the greatest influence on cheese. The fatty acids present in the same influence sensory parameters such as color, texture, and flavor (rancid and pungent). They likewise influence the nutritional composition of cheese as different fatty acids have beneficial or harmful effects on human health. On the other hand, the determination of the fatty acids present in cheese has been put forward as a useful tool for distinguishing the various cheeses according to the milk used in their production. Finding a tool which allows the determination of the fatty acids present in cheese in a rapid and non destructive manner is of great interest to the cheese industry. In this study we examine the use of Near-Infrared Spectroscopy (NIRS) technology in the determination of 19 fatty acids in cheese from C8:0 to C20:0 including ΣSFA and ΣUFA. Cheeses were made with known and varying percentages of cow, ewe's, and goat milk (112 samples) and ripening controls were carried out for 6 months. Two ways of recording the spectra are compared, one using a remote reflectance fiber-optic probe on a slice of cheese and another using the fatty extracts obtained from the same cheeses and recorded with cam-lock cells. The regression method used is MPLS. The results obtained reveal that it is possible to predict the fatty acid composition of cheese by means of the use of NIRS, irrespective of the method used to record it. Furthermore, the results obtained in the validation of the method used indicate that the equations obtained allow their application to unknown cheese samples.

1. Introduction

The characteristics presented by a cheese are influenced by the fatty acid profile of the milk used in its production [1]. The degree of unsaturation of fatty acids affects texture in cheese; higher unsaturation is correlated with a softer texture [2]. Regarding flavor, an increase in rancid flavor is related to the release of free fatty acids, which is mainly due to a higher amount of butyric acid [3], while a higher amount of short-chain fatty acids is associated with a more pungent flavor [4]. The changes in the fatty acid profile may sometimes be accompanied by an alteration of the flavor of the cheese [5]. The qualitative milk fat composition, especially the double bonds in a specific mixture of triglycerides, can be regarded as the most important descriptor of cheese body color [6] and the increase of fat lipolysis as ripening progresses intensify the yellow color.

From a nutritional point of view the determination of the fatty acid composition of cheese has attracted considerable interest because cheese is particularly rich in saturated fatty acids (SFAs), which is associated with an increase in cardiovascular risk, obesity, and some cancers [7]. However, only C12:0, C14:0, and C16:0 seem to be unhealthy while C18:0 has similar effects on cholesterol levels to oleic acid [8] and short-chain SFA has positive effects on human health. In fact, C4:0 shows positive effects in reducing cancer cell growth; C:6, C:8, and C10:0 have the potential to reduce body fat [9].

However, milk and dairy products are the main sources of conjugated linoleic acid (CLA), which has attracted increasing attention due to its health promoting biological properties.

On the other hand, Ha and Lindsay [10] suggested that the qualitative presence and quantitative amounts of milk fatty acids might contribute towards differentiating the cheese type. Moreover, goat and ewe's milk cheeses contain high levels of short- and medium-chain fatty acids compared to cow milk cheeses [11]. Some studies point out that the milk fatty acid profile could be a clue to distinguishing between breeds [12].

* Corresponding author.
E-mail addresses: inmaglez@usal.es (M.I. González-Martín), avivar@usal.es (A.M. Vivar-Quintana), irevilla@usal.es (I. Revilla), avivar@usal.es (J. Salvador-Esteban).

https://doi.org/10.1016/j.microc.2020.104854
Received 1 February 2020; Received in revised form 19 March 2020; Accepted 19 March 2020
Available online 20 March 2020
0026-265X/

As follows from all that has been expounded, it is important to characterize the fatty acid composition of cheese. The method used for fatty acid determination involves extraction by solvents, methylation, and analysis by gas chromatography, which is time-consuming and requires the use of pollutant solvents and sophisticated equipment. Near Infrared Spectroscopy (NIRS) technology allows the avoidance of all these disadvantages and is also a multiparametric method [13]. It has been used to determinate the physical-chemical parameters of cheeses [14], salts [15] and sensory properties [16]. The use of NIRS together with a fiber-optic probe without any sample preparation has been successfully applied to determine peptides, minerals, texture, and sensory properties and to analyze the percentage of milk of different animal species used in the production of cheeses with different ripening times [17,18,19,20]. NIR technology equipment has evolved in recent years towards small cheap NIR devices. Technological progress has made possible the development of small NIR equipment which is of particular interest to the agro-food industry [21]. Studies have been carried out [22,23,24] which reveal that the results obtained using portable devices are comparable to those achieved using bench-top spectrometers. The progressive reduction in size has led to the development of the pocket-sized NIR spectrometer, although its application to the study of food is still limited [25,26]. As far as cheese is concerned, the viability of using a pocket-sized molecular sensor. The SCiO (Consumer Physics, Tel Aviv, Israel) to predict the fat and moisture content [27] has been studied. The results obtained by these authors reveal the viability of the use of this equipment to determine both parameters.

Some studies have also shown that NIRS technology allows the prediction of the fatty acid composition of milk [28] and of fresh cheeses using Vis-NIRS spectrometry [29,30]. They use the NIRS, FT-MIR and Raman spectroscopy techniques to determine fatty acids in dairy products (butter and Cheddar cheese) with inconclusive results for cheese. Tao and Ngadi [31] and Tong Lei and Da-Wen Sun [32] carried out a revision of spectroscopic techniques (Near-Infrared Spectroscopy (NIRS), Fourier transform infrared (FTIR) spectroscopy, Raman spectroscopy (RS), nuclear magnetic resonance (NMR), magnetic resonance imaging (MRI), etc. to determine among other aspects the fat and fatty acids in dairy foods, in particular in milk and fresh cheese. There are however no references to its use in cheeses of variable composition and at varying levels of ripening.

The objective of the study is to quantify the fatty acids of cheeses of variable composition as to the origin of the milk (cow, ewe's, and goat) and with different ripening times (up to 6 months) by using near-infrared spectroscopy. Two NIR methods will be used by applying a remote reflectance fiber-optic probe directly to a slice of cheese and by measuring the fat extracts obtained from the same cheeses with cam-lock cells. The two procedures are compared so as to assess their suitability. The reference method used in the quantification of fatty acids in cheese was gaseous chromatography.

2. Material and methods

2.1. Samples

112 cheeses were produced and analyzed using raw milk from farms located in Zamora, Spain; there were two production sets for each type of cheese (summer and winter). The cheeses were produced using different percentages of ewe's, goat, and cow milk (Table 1) as has been described by González-Martín et al. [33]. The cheeses were analyzed throughout their ripening period with samples being taken of each cheese type produced every month for the first 6 months. The analyses were carried out on a piece 2 cm high cut from the central area of the cheese.

In this study, 100 samples were used in the calibration set and 12 in the external validation set, both in the determination of fatty acids in the samples of cheese in slices and in the fat extracts obtained from these cheeses. The distribution of the samples in the two sets was carried out at random.

Table 1
The chemical composition of fatty acids of cheeses (mg FA/ g cheese) obtained by gas chromatography.

	Samples of cheese N = 112
C8:0	4.49 ± 1.55
C9:0	0.04 ± 0.07
C10:0	14.97 ± 4.97
C11:0	0.13 ± 0.05
C12:0	8.04 ± 1.90
C13:0	0.18 ± 0.08
C14:1	0.96 ± 0.56
C14:0	23.07 ± 4.69
C15:0	1.35 ± 0.61
C16:1	2.50 ± 0.67
C16:0	73.75 ± 19.02
C17:1	0.50 ± 0.21
C17:0	0.96 ± 0.33
C18:2	6.15 ± 1.71
C18:1 cis	38.62 ± 7.93
C18:1 trans	5.57 ± 3.95
C18:1 total	50.40 ± 11.29
C18:0	27.36 ± 7.38
C19:0	0.06 ± 0.08
C20:0	0.29 ± 0.14
ΣSFA	154.69
ΣUFA	54.35

2.2. Fatty acid analysis

Lipids were extracted using the International Standard Method described in ISO 14156:2001. The fatty acids (FAs) of all samples were methylated and analyzed by gas chromatography according to the method described by Soto-Barajas et al. [34]. An analysis was performed on a sample microliter which was injected in a gas chromatograph (GC 6890N, Agilent Technologies, USA) and equipped with a split/splitless injector and a flame ionization detector (FID). The FAs were identified by their retention times and their comparison with standards (Sigma Aldrich, Steinheim, Germany). The results obtained are expressed in mg FA/g cheese (Table 1).

2.3. NIR Spectroscopy

The reflectance spectra of cheeses are obtained by using a Foss-NIRSystems 5000 monochromator (Foss NIRSystems, Silver Spring, MD). When intact cheese samples are used records are made by using a 1.5 m 210/210 remote reflectance bundle fiber-optic probe (Ref. R6539-A) applied directly to the cheese slice as has been described by González-Martín et al. [35]. The samples are scanned using the quartz window of the probe with a surface area of 5 cm × 5 cm, registering from 1100 to 2000 nm every 2 nm (spectral bandpass 10 nm ± 1 nm), with which 450 pieces of data are obtained per sample. When work is carried out with samples of fat extracts the samples are scanned by using circular cells known as “cam-lock” cells with an optic passage of 0.1 nm used for pasty liquids; the analysis was performed using a transport module. Measurements in reflectance mode are taken from 1100 to 2498 nm every 2 nm (699 data points) per sample of fat extracted. Each spectrum represents the average of 3 registers. WINISI 1.5 (Infrasoft International, State College, PA) was used to obtain calibration equations.

2.4. Chemometric techniques

The dispersion phenomena of the signs of reflectance and undesirable contributions present the NIR signs are minimized with different spectral pre-treatments: derived and smoothed. All calibrations were

Table 2
Statistical descriptors of the calibration of fatty acids in intact cheese samples. NIR register with fiber-optic probe on a slice of cheese.

Component	N	Mathematical treatment	Mean	SD	Est. Min	Est. Max	SEC	SECV	RSQ	RPD
C8:0	95	Standard MSC 2,4,4,1	4.42	1.37	0.30	8.54	0.59	0.89	0.81	2.44
C9:0	93	Standard MSC 2,10,10,1	0.03	0.06	0.00	0.21	0.04	0.05	0.54	1.19
C10:0	94	SNV 2,4,4,1	14.65	4.60	0.84	28.45	1.66	2.57	0.87	2.94
C11:0	93	Ninguno 1,4,4,1	0.12	0.05	0.00	0.26	0.03	0.04	0.52	1.17
C12:0	92	Standard MSC 1,4,4,1	7.90	1.67	2.88	12.93	0.84	1.09	0.75	2.10
C13:0	92	Standard MSC 2,8,6,1	0.18	0.07	0.00	0.38	0.04	0.05	0.72	1.98
C14:1	93	Standard MSC 2,4,4,1	0.89	0.46	0.00	2.28	0.19	0.35	0.84	2.65
C14:0	93	Detrend 0,0,1,1	22.72	4.45	9.38	36.07	2.70	2.96	0.63	1.74
C15:0	93	SNV 2,8,6,1	1.28	0.55	0.00	2.94	0.35	0.44	0.60	1.67
C16:1	95	SNV 2,8,6,1	2.45	0.62	0.59	4.31	0.42	0.54	0.54	1.82
C16:0	95	Standard MSC 1,4,4,1	72.37	13.86	30.79	113.95	9.16	11.34	0.56	1.59
C17:1	94	SNV 2,4,4,1	0.48	0.14	0.07	0.89	0.08	0.12	0.68	1.87
C17:0	92	Standard MSC 1,4,4,1	0.91	0.19	0.33	1.50	0.13	0.15	0.57	1.59
C18:2	94	Ninguno 2,4,4,1	6.08	1.67	1.08	11.08	0.73	1.04	0.81	2.39
C18:1 cis	95	SNV 2,4,4,1	38.41	7.90	14.71	62.12	3.54	5.70	0.80	2.36
C18:1 trans	94	SNV 2,4,4,1	5.15	2.21	0.00	11.77	0.95	1.41	0.81	2.46
C18:1 total	93	Standard MSC 1,4,4,1	49.53	10.11	19.22	79.85	5.59	6.87	0.69	1.90
C18:0	89	Standard MSC 0,0,1,1	26.29	6.80	5.88	46.70	3.05	3.53	0.80	2.37
C20:0	95	Detrend 2,4,4,1	0.28	0.13	0.00	0.67	0.09	0.12	0.57	1.59
ΣSFA	94	Standard MSC 1,4,4,1	152.01	30.95	59.15	244.86	16.75	21.72	0.71	1.96
ΣUFA	93	SNV 1,4,4,1	53.44	10.68	21.40	85.48	6.14	7.49	0.67	1.83

obtained for the NIR spectral range (1100–2498 nm) for each parameter when the samples are of extracted fat and for the 1100–2000 nm range when the samples are of cheese slices. The number of variables is reduced by principal component analysis (PCA). The quantification of the FA was performed by the MPLS regression method. In MPLS the NIR residuals at each wavelength, obtained after each factor has been calculated, are standardized (by dividing the standard deviations of the residuals at each wavelength) before calculating the next factor. For cross-validation, the calibration set was partitioned in groups (7 in the cheese samples and 6 for the fat extract samples); each group was then predicted using calibration developed on the other samples; finally, validation errors were combined to obtain a standard error of cross-validation (SECV). The best calibrations were selected based on the higher *R* squared, RSQ, multiple correlation coefficient values, and lower SECV.

3. Results and discussion

3.1. Chemical analyses

Table 2 shows the average concentration and the standard deviations of FAs (from C:8 to C:20, ΣSFA and ΣUFA). The composition of the fatty acids in cheese mainly depends on the FA content of the raw milk [36] and their FA profile does not substantially change due to the processing [37]. The fatty acids most commonly analyzed in the cheese samples were C10:0, C14:0, C16:0, C18:1 *cis*, and C18:0. The concentration of monounsaturated fatty acids may range from about 20% to about 35% and is similar in ewe's, cow, and goat milk fat [38]. Oleic acid (C18:1) is the most frequent monounsaturated fatty acid in all ruminant milk [39,40], although its proportion in cow milk is higher than that described for goat and ewe's milk [41]. The percentage of saturated fatty acids varies between 60% and 70% of total fatty acids in ruminant milk, and palmitic acid makes the highest contribution to this percentage [38].

The most noteworthy difference between cow milk compared with goat and ewe's milk is the high amount of medium-chain fatty acids contained in ewe's and goat milk, particularly of C6:0, C8:0, and C10:0 fatty acids, which is revealed in the high values of typical deviation (Table 2). These fatty acids have been associated with the specific milk aroma of these small ruminants; even the lauric C12:0 and capric C10:0 acids relation is being used as an indicator for detecting falsifications of goat milk using cow milk [39].

In addition to the fatty acid composition of milk, the period of the ripening of the cheese contributes to the modification of its lipid profile. An increase in medium-chain saturated FAs and a decrease in several polyunsaturated FAs has been observed during the first period (6 months), but not during the last period of the one year ripening process [42].

The fatty acid composition of cheese depends on dietary factors, breeds, ripening times, regions, and environmental factors. It is for this reason that any proposal for a rapid methodology to determine these compounds must include a wide representation of the fatty acids present in cheese. The cheeses analyzed in this study are sufficiently varied to allow appropriate calibration to be carried out.

3.2. Spectral characteristics

The study is divided into two parts referring to whole samples and to those extracted separately. Fig. 1 shows the NIR spectra of the samples depending on the recording mode.

The absorption bands are characteristics of the different bonds and they are reflected in the spectral information. The C—H bond, which is fundamental in fatty acids, shows strong absorbance at wavelengths of approximately 1200, 1400, 1750, 2310, and 2340 nm. The combination bands of this bond show a characteristic absorbance in the 2310–2340 region while the first overtone of the C—H band is responsible for the absorption observed in the 1720–1760 region [43]. The CH_2 band, and especially its second overtone, strongly absorbs at 1210 nm [44]. Other authors [45] related the absorption observed at 1680 nm and in the 2150–2190 interval to the presence of unsaturated fatty acids with cis double bonds. Moreover, according to Hourant et al [46], the observed band near to 1725 is related to oleic acid. Finally, Šašić and Ozaki [47] consider that in dairy products wavelengths of 1760 nm are allocated to the components of saturated acids and of 2030 and 2380 nm to the vibrations of the fats. The contribution to lipids can also be observed at 1765–1730 nm, which was associated with C═O stretching of carbonyl groups of acids and esters [48].

3.3. Fatty acid composition quantification by means of NIR

3.3.1. Calibration equations

An analysis of major components has been carried out on the samples. The spectral variability explained was higher than 98%, with between 7 and 9 major components being needed when working with

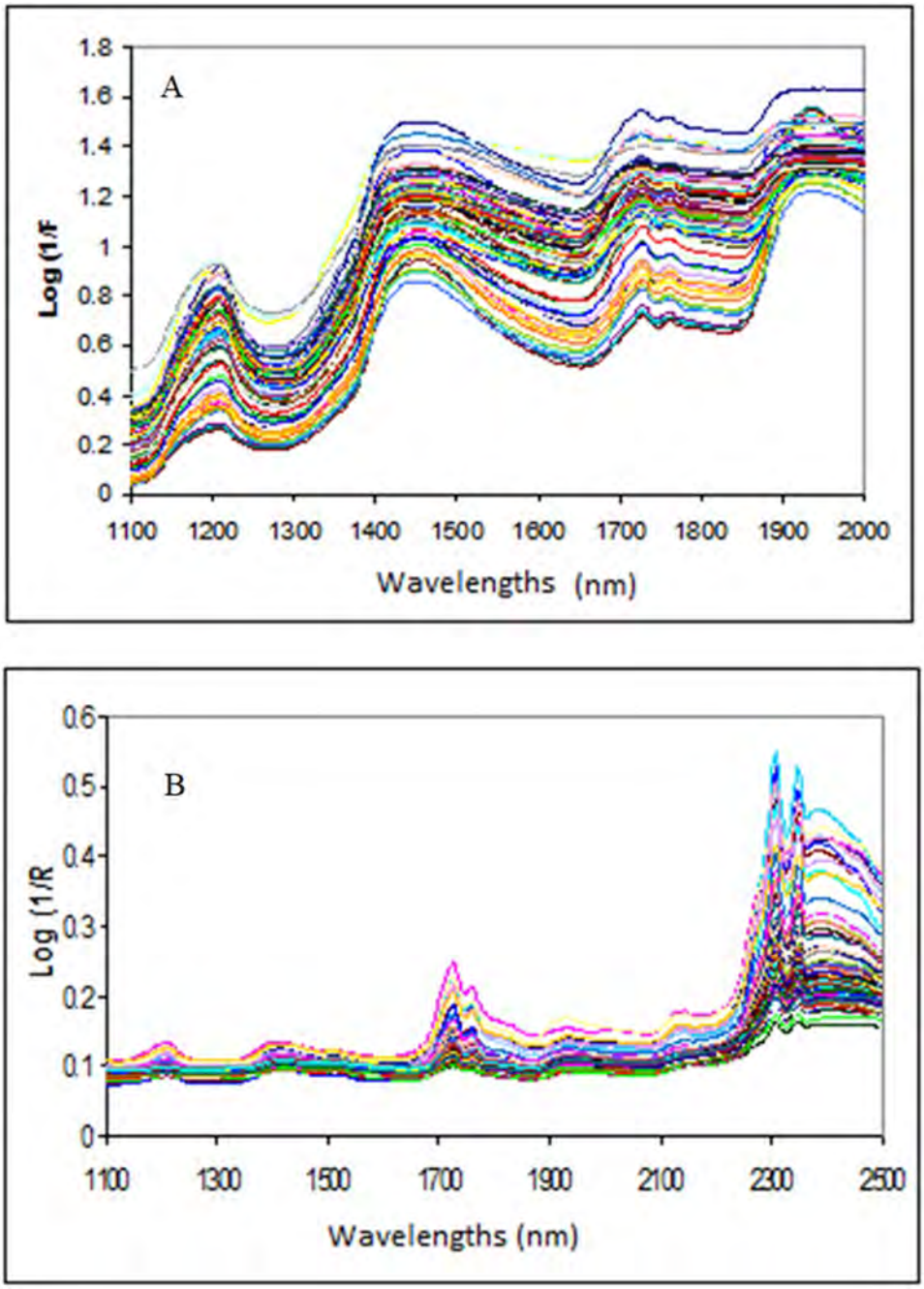

Fig. 1. NIR spectra obtained by using a remote reflectance fiber-optic probe in intact samples (A) and cam-lock cells for extracted samples (B).

whole samples and between 6 and 8 when the fat samples extracted are used. The statistical parameters of the MPLS calibration (Rˆ2, SEC, SECV and RPD) are shown in Table 3 (intact samples) and in Table 4 (for liquid samples of fat extracted from the cheeses). The multiple scatter correction (MSC), the Standard Normal Variat (SNV), Detrend (DT) or SNV-DT were used to remove the effects of dispersion.

The Mahalanobis distance (H) as a spectral criterion (H) and the chemical criterion (T) were used to remove some outlier samples. Tables 2 and 3 show the final number of samples included in the model (N) together with the best optimized treatment.

The NIR equations, which allow the determination of 19 fatty acids (all those which are determined by means of gaseous chromatography except C19:0) both in cheese samples registered as whole and in those registered in the form of fat extracts, have good statistical descriptors of calibration. It should be stressed that the margins of application of these mathematical models for the determination of fatty acids in intact samples are comparable with those obtained by chromatography. However, when the fat extracts obtained from the same cheeses are used, in general the margins allowing the determination of the fatty acids are narrower and the RSQ values exceed those obtained by using registers with a fiber-optic probe.

3.3.2. *Internal validation*

Model assessment was carried out by cross-validation. To do this, the set of calibration samples is divided into 7 subsets (intact samples of cheese) or 6 subsets (fat samples). Of these, 6 subsets for intact samples or 5 subsets for fat samples are taken for calibration and the other subset is the prediction set. To allow all sets to undergo the calibration and prediction process, this procedure was repeated 6 or 7 times. The results reflect the validity and the prediction capacity of the model.

Table 3
Statistical descriptors of the calibration of fatty acids in samples of extracted fat. NIR register obtained with cam-lock cells.

Component	N	Mathematical treatment	Mean	SD	Est. Min	Est. Max	SEC	SECV	RSQ	RPD
C8:0	91	SNV 2,8,6,1	1.75	0.3	0.89	2.62	0.12	0.18	0.84	1.40
C9:0	93	Detrend 0,0,1,1	0.01	0.0	0.00	0.09	0.01	0.02	0.77	2.40
C10:0	90	Standard MSC 2,4,4,1	6.14	1.2	2.61	9.67	0.29	0.69	0.94	2.22
C11:0	92	SNV	0.09	0.0	0.01	0.16	0.01	0.02	0.76	2.43
C12:0	95	None 2,4,4,1	4.34	0.3	3.53	5.16	0.11	0.24	0.84	1.12
C13:0	92	SNV 2,8,6,1	0.11	0.0	0.00	0.23	0.02	0.03	0.83	1.26
C14:1	91	SNV 1,4,4,1	0.44	0.3	0.00	1.20	0.09	0.14	0.88	1.70
C14:0	93	Standard MSC 1,4,4,1	10.99	0.7	8.89	13.08	0.37	0.62	0.72	1.68
C15:0	94	Standard MSC 2,8,6,1	1.28	0.3	0.24	2.32	0.12	0.21	0.88	1.69
C16:1	90	SNV 2,8,6,1	1.44	0.3	0.50	2.37	0.11	0.18	0.86	1.06
C16:0	90	Standard MSC 0,0,1,1	30.98	2.2	24.41	37.55	1.21	1.39	0.69	1.83
C17:1	92	Standard MSC 1,4,4,1	0.22	0.0	0.07	0.37	0.02	0.03	0.87	1.51
C17:0	93	None 2,8,6,1	0.68	0.1	0.41	0.96	0.05	0.07	0.75	1.86
C18:2	92	SNV 1,4,4,1	3.44	0.6	1.54	5.35	0.23	0.34	0.87	1.40
C18:1 cis	94	SNV 2,8,6,1	19.91	1.4	15.74	24.09	0.59	0.80	0.82	1.54
C18:1 trans	91	Detrend 0,0,1,1	3.06	1.1	0.00	6.37	0.57	0.70	0.73	1.40
C18:1 total	90	SNV 2,4,4,1	23.03	1.0	20.04	26.03	0.55	1.07	0.70	2.37
C18:0	92	Standard MSC 1,4,4,1	14.74	1.7	9.68	19.81	0.62	1.04	0.86	2.22
C20:0	94	Standard MSC 1,4,4,1	0.20	0.0	0.07	0.34	0.02	0.03	0.77	1.12
ΣSFAo	89	None 2,4,4,1	71.42	1.2	67.68	75.15	0.57	1.07	0.79	1.26
ΣUFA	89	None 2,4,4,1	28.58	1.2	24.85	32.32	0.57	1.07	0.79	1.70

Table 4
External validation of fatty acids in cheese samples. Application to 12 samples. NIR register using a fiber-optic probe on cheese.

Component	RMSE	P (confidence level)
C8:0	0.16	0.94
C9:0	0.01	0.66
C10:0	0.74	0.33
C11:0	0.01	0.51
C12:0	0.28	0.72
C13:0	0.02	0.12
C14:1	0.12	0.24
C14:0	0.92	0.51
C15:0	0.18	0.18
C16:1	0.18	0.91
C16:0	2.53	0.96
C17:1	0.05	0.17
C17:0	0.10	0.38
C18:2	0.26	0.97
C18:1 cis	1.55	0.78
C18:1 trans	0.44	0.23
C18:1 total	1.84	0.89
C18:0	1.01	0.60
C20:0	0.02	0.56
ΣSFA	4.36	0.87
ΣUFA	2.21	0.94

Fig. 2 shows the correlation between the reference and the predicted values obtained by NIR spectroscopy, comparing the results obtained with the two forms of register for some FAs.

The performance of the models was determined by the RSQ coefficient (the squared correlation coefficient) for reference versus predicted values in cross-validation. Another parameter which provides information on the performance of the model is the RPD (the ratio of performance to deviation) which is calculated as the SD quotient divided by SECV. The reference value for a good calibration is a RPD value higher than 2. SD, SECV and RPD are shown in Tables 2 and 3, in which it can be observed that the best models obtained by using RPD parameters would be those corresponding to the fiber-optic probe, except for the fatty acids C9:0, C11:0, C16:0, C17:0, and C18:1.

Taking into account these results, NIR spectroscopy coupled with a fiber-optic probe is a viable alternative for the determination of the fatty acid percentage in different types of cheese without either treating or manipulating the cheese sample,

3.3.3. External validation

To check the efficiency of the equations obtained by NIR spectroscopy, 12 new samples from the external calibration set were used to compare the reference data with the predicted values obtained. As these new samples do not belong to the calibration set, they have different spectral curves. Table 4 shows the results of the Student's *t*-test for paired values when reference data and NIRS values of the validation set samples were compared. The p values were lower than 0.05 for all the fatty acids determined; there were not therefore significant differences between the values provided by both methods. The RMSEs (Root Mean Standard Errors) fell within the interval between 0.02 for C20:0 and 4.32 for ΣSFA. These results allow us to conclude that the prediction equations were satisfactory for the fatty acids showed in Table 4.

4. Conclusion

This study confirms the viability of NIRS as a rapid, reliable, and efficient analytical method for providing information on the lipid profile of cheese samples. The variability of the cheeses studied, the use of cow, ewe's, and goat milk in their production, and the period of time of the ripening of the cheeses analyzed allow us to conclude that the methodology, once perfected, can be successfully applied to samples of unknown origin. The results of the determination of 19 fatty acids from C8:0 to C20:0, ΣSFA, and ΣUFAs in cheeses were compared by using a remote reflectance fiber-optic probe directly on the cheese and recorded with cam-lock cells from fat extracts obtained from the same cheeses; it can be concluded that both methods give good results. On the other hand, recording on a slice of cheese in a direct manner allowed the obtaining of better results for some fatty acids, which together with the fact that handling the sample is unnecessary appears to indicate that it is the best alternative for the obtaining of NIR spectra.

CRediT authorship contribution statement

M. Inmaculada González-Martín: Methodology, Writing - original draft. **Ana M. Vivar-Quintana:** Conceptualization, Writing - review & editing. **Isabel Revilla:** Investigation, Validation. **Javier Salvador-Esteban:** Formal analysis.

Declaration of Competing Interests

The authors declare that they have no known competing financial

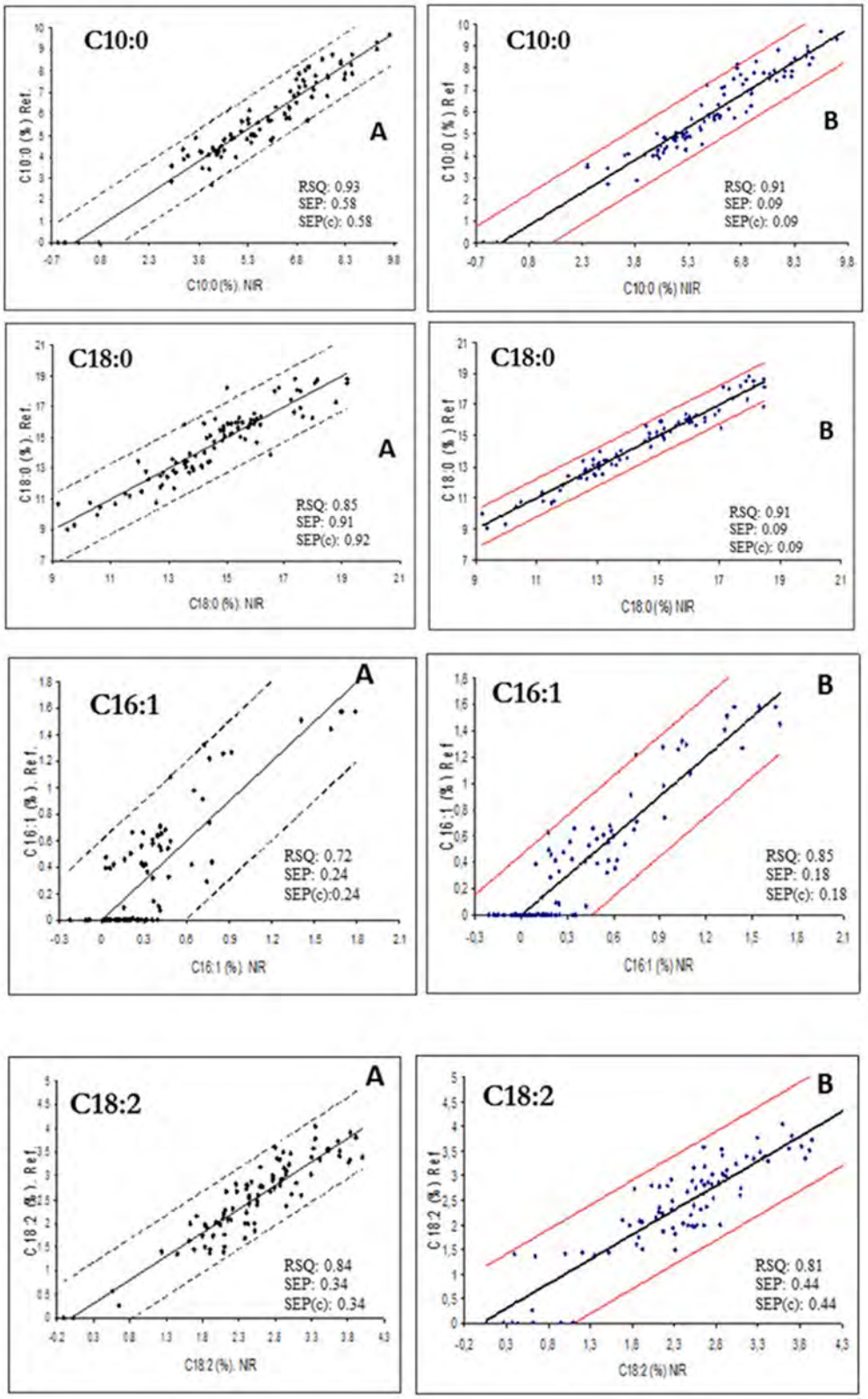

Fig. 2. Comparison of reference values with predicted values by the NIRS model for fatty acids with the recording mode of the samples. (A): register using a fiber-optic probe; (B): register with cam-lock cells (RSQ, multiple correlation coefficient, SEP, square error of prediction, SEP(C), standard error of prediction corrected by BIAS).

interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgment

The authors are grateful for funding for Project CTQ200604383, enabling this work to be carried out.

This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

References

[1] S. Buchin, V. Delague, G. Duboz, J.L. Berdague, E. Beuvier, S. Pochet, R. Grappin, Influence of pasteurization and fat composition of milk on the volatile compounds and flavor characteristics of a semi-hard cheese, J. Dairy Sci. 81 (1998) 3097–3108, https://doi.org/10.3168/jds.S0022-0302(98)75874-6.

[2] C. Bugaud, S. Buchin, Y. Noël, L. Teissier, S. Pochet, B. Martin, J.F. Chamba, Relationships between abondance cheese texture, its composition and that of milk produced by cows grazing different types of pastures, Le Lait 81 (2001) 593–607 10.1051/lait:2001152.

[3] Y.F. Collins, P.L.H. Mc Sweeney, M.G. Wilkinson, Lipolysis and free fatty acid catabolism in cheese: a review of current knowledge, Int. Dairy J. 13 (2003) 841–866, https://doi.org/10.1016/S0958-6946(03)00109-2.

[4] M. Gobetti, R. Lanciotti, M. De Angelis, M.R. Corbo, R Massini, P.F. Fox, Study of the effects of temperature, pH, NaCl, and aw in the proteolytic and lipolytic activities of cheese-related lactic acid bacteria by quadratic response surface methodology, Enzyme Microb. Technol. 25 (1999) 795–809, https://doi.org/10.1016/S0141-0229(99)00110-6.

[5] Chilliard, Y., Rouel, J., Ferlay, A., Bernard, L., Gaborit, P., Raynal-Ljutovac, K. & Lauret, A. (2005). Effects of type of forage and lipid supplementation on goat milk fatty acids and sensorial properties of cheeses. In International Dairy Federation (Ed.), The Future of the Sheep and Goat Dairy Sector (pp. 297–304).

[6] H. Rohm, D. Jaros, Colour of hard cheese. Factors of influence and relation to compositional parameters. Zeitschrift für Lebensmittel-Untersuchung und Forschung 204 (1997) 259–264, https://doi.org/10.1007/s002170050074.

[7] J.D. Wood, R.I. Richardson, G.R. Nute, A.V. Fisher, M.M. Campo, P.R. Kasapidou, M. Enser, Effects of fatty acids on meat quality: a review, Meat Sci. 66 (1) (2004) 21–32, https://doi.org/10.1016/S0309-1740(03)00022-6.

[8] R.P. Mensink, Effects of stearic acid on plasma lipid and lipoproteins in humans, Lipids 40 (2005) 1201–1205, https://doi.org/10.1007/s11745-005-1486-x.

[9] J.B. German, C.J. Dillard, Composition, structure and absorption of milk lipids. A source of energy, fat-soluble nutrients and bioactive molecules, Crit. Rev. Food Sci. Nutr. 46 (2006) 57–92, https://doi.org/10.1080/10408690590957098.

[10] J.K. Ha, R.C. Lindsay, Release of volatile branched-chain and other fatty acids from ruminant milk fats by various lipases, J. Dairy Sci. 76 (1993) 677–690, https://doi.org/10.3168/jds.S0022-0302(93)77391-9.

[11] S. Gnädig, J.F. Chamba, E. Perreard, S. Chappaz, J.M. Chardigny, R. Rickert, H. Steinhart, J.L. Sebedio, Influence of manufacturing conditions on the conjugated linoleic acid content and the isomer composition in ripened French Emmental cheese, J. Dairy Res. 71 (2004) 367–371, https://doi.org/10.1017/S0022029904000226.

[12] F. Signorelli, G. Contarini, G. Annicchiarico, F. Napolitano, L. Orrù, G. Catillo, G.F.W. Haenlein, B. Moioli, Breed differences in sheep milk fatty acid profiles: opportunities for sustainable use of animal genetic resources, Small Rumin. Res. 78 (2008) 24–31, https://doi.org/10.1016/j.smallrumres.2008.04.003.

[13] J.L. Rodriguez-Otero, M. Hermida, J. Centeno, Analysis of dairy products by near-infrared spectroscopy: a review, J. Agric. Food Chem. 45 (1997) 2815–2819, https://doi.org/10.1021/jf960744p.

[14] D. McKenna, Measuring moisture in cheese by near-infrared absorption spectroscopy, J. AOAC Int. 84 (2001) 623–628.

[15] C. Blaquez, G. Downey, C. O'Donnell, D. O'Callaghan, V. Howard, Prediction of moisture, fat and inorganic salts in processed cheese by near infrared reflectance spectroscopy and multivariate data analysis, J. Near Infrared Spectrosc. 12 (2004) 149–157, https://doi.org/10.1255/jnirs.420.

[16] G. Downey, E. Sheehan, C. Delahunty, D. O'Callaghan, T. Guinee, V. Howard, Prediction of maturity and sensory attributes of Cheddar cheese using near-infrared spectroscopy, Int. Dairy J. 15 (2005) 70–709, https://doi.org/10.1016/j.idairyj.2004.06.013.

[17] M.I. González-Martín, J.M. Hernández-Hierro, R. Morón-Sancho, J. Salvador-Esteban, A.M. Vivar-Quintana, I. Revilla, Determination of the percentage of milk (cow's, ewe's and goat's) in cheeses with different ripening times using NIRS technology and a remote reflectance fibre-optic probe, Anal. Chim. Acta 604 (2007) 191–196, https://doi.org/10.1016/j.aca.2007.10.014.

[18] M.I. González-Martín, J.M. Hernández-Hierro, I. Revilla, A.M. Vivar-Quintana, C. González-Pérez, The application of near infrared spectroscopy technology and a remote reflectance fibre-optic probe for the determination of peptides in cheeses (cow's, ewe's and goat's) with different ripening times, Food Chem. 114 (2009) 1564–1569, https://doi.org/10.1016/j.foodchem.2008.11.050.

[19] I. González-Martín, J.M. Hernández-Hierro, I. Revilla, A.M. Vivar-Quintana, I. Lobos Ortega, The mineral composition (Ca, P, Mg, K, Na) in cheeses (cow's, ewe's and goat's) with different ripening times using near infrared spectroscopy with a fibre-optic probe, Food Chem. 127 (2011 a) 147–152, https://doi.org/10.1016/j.foodchem.2010.12.114.

[20] I. Revilla, M.I. González- Martín, J.M. Hernández Hierro, A.M. Vivar-Quintana, C. González-Pérez, M.A. Lurueña-Martínez, Texture evaluation in cheeses by NIRS technology employing a fibre-optic probe, J. Food Eng. 92 (2009) 24–28, https://doi.org/10.1016/j.jfoodeng.2008.10.022.

[21] C.A. dos Santos, M. Lopo, R.N. Páscoa, J.A. Lopes, A review on the applications of portable near-infrared spectrometers in the agro-food industry, Appl. Spectrosc. 67 (2013) 1215–1233, https://doi.org/10.1366/13-07228.

[22] R. Beghi, A. Spinardi, L. Bodria, I. Mignani, R. Guidetti, Apples nutraceutic properties evaluation through a visible and nearinfrared portable system', Food Bioprocess Technol. 6 (2013) 2547–2554.

[23] J.A. Cayuela, C. Weiland, Intact orange quality prediction with two portable NIR spectrometers, Postharvest Biol. Technol. 58 (2010) 113–120.

[24] D. Pérez-Marín, P. Paz, J.E. Guerrero, A. Garrido-Varo, M.T. Sánchez, Miniature handheld NIR sensor for the on-site nondestructive assessment of post-harvest quality and refrigerated storage behavior in plums, J. Food Eng. 99 (2010) 294–302.

[25] H. Kaur, R. Künnemeyer, A. McGlone, Comparison of hand-held near infrared spectrophotometers for fruit dry matter assessment, J. Near Infrared Spectrosc. 25 (2017) 267–277, https://doi.org/10.1177/0967033517725530.

[26] H Yan, H.W Siesler, Hand-held near-infrared spectrometers:State-of-the-art instrumentation and practical applications, NIR News 29 (7) (2018) 8–12, https://doi.org/10.1177/0960336018796391.

[27] V. Wiedemair, D. Langore, R. Garsleitner, K. Dillinger, C.W. Huck, Investigations into the performance of a novel pocket-sized near-infrared spectrometer for cheese analysis, Molecules 24 (2019) 428–441, https://doi.org/10.3390/molecules24030428.

[28] M. Coppa, A. Ferlay, C. Lerouz, M. Jestin, Y. Chilliard, B. Martin, D. Andueza, Prediction of milk fatty acid composition by near infrared reflectance spectroscopy, Int. Dairy J. 20 (2010) 182–189, https://doi.org/10.1017/S1751731111000747.

[29] A. Lucas, D. Andueza, A. Ferlay, B. Martin, Prediction of fatty acid composition of fresh and freeze-dried cheeses by visible-near infrared reflectance spectroscopy, Int. Dairy J. 18 (2008) 595–604, https://doi.org/10.1016/j.idairyj.2007.12.001.

[30] M. Zhao, J.R. Beattie, A.M. Fearon, C.P. O'Donnell, G. Downey, Prediction of naturally-occurring, industrially-induced and total trans fatty acids in butter, dairy spreads and Cheddar cheese using vibrational spectroscopy and multivariate data analysis, Int. Dairy J. 51 (2015) 41–51, https://doi.org/10.1016/j.idairyj.2015.07.011.

[31] F. Tao, M. Ngadi, Applications of spectroscopic techniques for fat and fatty acids analysis of dairy foods, Curr. Opin. Food Sci. 17 (2017) 100–112, https://doi.org/10.1016/j.cofs.2017.11.004.

[32] L. Tong, S. Da-Wen, Developments of nondestructive techniques for evaluating quality attributes of cheeses: a review, Trends Food Sci. Technol. 88 (2019) 527–542, https://doi.org/10.1016/j.tifs.2019.04.013.

[33] M. González-Martín, V. Vicente Palacios, I. Revilla, A.M. Vivar-Quintana, J.M. Hernández-Hierro, Discrimination between cheeses made from cow´s, ewe´s and goat´s milk from unsaturated fatty acids and use of the canonical biplot method, J. Food Compos. Anal. 56 (2017) 34-40.10.1016/j.jfca2016.12.005.2010.12.105.

[34] M.C. Soto-Barajas, I. González-Martín, J. Salvador-Esteban, J.M. Hernandez-Hierro, V. Moreno-Rodilla, A.M. Vivar Quintana, I. Revilla, I. Lobos Ortega, R. Morón-Sancho, B. Curto-Diego, Prediction of the type of milk and degree of ripening in cheese by means of artificial neural networks with data concerning fatty acids and near infrared spectroscopy, Talanta 116 (2013) 50–55, https://doi.org/10.1016/j.talanta.2013.04.043.

[35] I. González-Martín, J.M. Hernández-Hierro, J. Salvador-Esteban, C. González-Pérez, I. Revilla, A.M. Vivar-Quintana, Discrimination of seasonality in cheese by near-infrared technology, J. Sci. Food Agric. 91 (2011 b) 1064–1069, https://doi.org/10.1002/jsfa.4283.

[36] A. Nudda, M.A. McGuire, G. Battacone, G. Puina, Seasonal variation in conjugated linoleic acid an vaccenic acid in milk fat of sheep and its transfer of cheese and ricotta, J. Dairy Sci. 88 (2005) 1311–1319, https://doi.org/10.3168/jds.S0022-0302(05)72797-1.

[37] A. Nudda, G. Battacone, O. Boaventura Neto, A. Cannas, A.H. Dias Francesconi, A. Stanislao Alzori, G. Pulina, Feeding strategies to design the fatty acid profile of sheep milk and cheese, Rev. Brasileira Zootecnia 43 (2014) 445–456, https://doi.org/10.1590/S1516-35982014000800008.

[38] M. Markiewicz-Keszycka, G. Czyżak-Runowska, P. Lipińska, Jacek Wójtowski, Fatty acid profile: a review, Bull. Vet. Inst. Pulawy 57 (2013) 135–139, https://doi.org/10.2478/bvip-2013-0026.

[39] N. Strzałkowska, A. Jóźwik, E. Bagnicka, J. Krzyżewski, K. Horbańczuk, B. Pyzel, J.O. Horbańczuk, Chemical composition, physical traits and fatty acid profile of goat milk as related to the stage of lactation, Anim. Sci. Pap. Rep. 27 (2009) 311–320.

[40] A. Anghel, D. Nadolu1, A. Sava, A. Cismileanu, M. Ropota, I. Voicu, Chemical composition and fatty acid profile of Carpathian goat milk as related to the stage of lactation, Archiva Zootechnica 20 (2017) 59–68.

[41] M. Szumacher-Strabel, A. Cieślak, P. Zmora, E. PersKamczyc, S. Bielińska, M. Stanisz, J. Wojtowski, Camelina sativa cake improved unsaturated fatty acids in ewe's milk, J. Sci. Food Agric. 91 (2011) 2031–2037, https://doi.org/10.1002/jsfa.4415.

[42] M. Bergamaschi, G. Bittante, Delaited fatty acid profile of milk, cheese ricotta and by products from cows grazing summer highland pastures, J. Dairy Res. 84 (2017) 329–338, https://doi.org/10.1017/S0022029917000450.

[43] J.S. Shenk, M.O. Westerhaus, Routine operation, calibration, development and network system management manual, Maryland (1995).

[44] B. Osborne, T. Fearn, P. Hindle, Practical NIR Spectroscopy with Applications in Food and Beverage Analysis: Longman Scientific and Technical, Harlow, UK, 1993.
[45] A. Garrido-Varo, R. Carrete, J. Fernandez-Cabanás, Use of difference near infrared reflectance spectra to extract relevant information from the spectra of agro-food products, J. Near Infrared Spectrosc. 6 (1998) 89–95, https://doi.org/10.1255/jnirs.125.
[46] P. Hourant, V. Baeten, M. Morales, M. Meurens, R. Aparicio, Oil and fat classification by selected bands of near-infrared spectroscopy, Appl. Spectrosc. 54 (2000) 1168–1174, https://doi.org/10.1366/0003702001950733.
[47] S. Šašić, Y. Ozaki, Band assignment of near-infrared spectra of milk by use of partial least-squares regression, Appl. Spectrosc. 54 (2000) 1327–1338, https://doi.org/10.1366/0003702001951002.
[48] A. Subramanian, V. Prabhakar, L. Rodriguez-Saona, Infrared spectroscopy in dairy analysis, in: J.W. Teoksessa Fuquay, P.F. Fox, P.L.H. McSweeney (Eds.), Encyclopedia of Dairy Sciences, Elsevier Academic Press, 2011, pp. 115–124.