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Near-infrared spectroscopy to assess chemical composition of sheep and goat cheeses

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The present study aimed to evaluate the performances of Fourier transform near-infrared spectroscopy technique to determine the chemical and the fatty acid composition of different types of cheeses. A total of 95 cheeses from sheep and goat raw milk were produced in small local dairies of Siena province (Tuscany). For each cheese, spectrum was collected in intact slices of the sample and fatty acid profile was determined in ground samples. Outliers were identified and different mathematical pre-processing treatments (SNV, MSC, baseline correction and de-trending) were applied when necessary. Considering traditional chemical analysis and raw cheese spectral data, calibration and cross-validation models were carried out using partial least squares regression (PLS). The best results were evaluated in terms of coefficient of determination in calibration and cross-validation (R^2cv), and root mean square error in calibration and cross-validation, and residual prediction deviation (RPD). Moisture, protein and ash showed the best R^2cv (0.89, 0.74 and 0.72, respectively) and RPD values (3.0, 2.6 and 2.1, respectively). Saturated, monounsaturated and polyunsaturated fatty acids showed R^2cv which ranged from 0.75 to 0.67, and RPD <2.0. Intermediate results in terms of R^2cv (0.62 as mean) were obtained for medium chain saturated fatty acids (C8:0 to C14:0), whereas for C18 series only oleic acid reached good accuracy of prediction (R^2cv >0.70). Obtained results are promising and additional samples could strongly increase the predictive ability for small dairy farms.

Keywords: FT-NIRS, cheese, fatty acid, quality

1 Introduction

Cheese is a complex mixture of water, protein, fat, ions and various chemical compounds. During its maturation or ripening phase, cheese undergoes a complex series of chemical, bacterial and enzymatic reactions which are responsible for the breakdown of the protein matrix and definitively of the development of the texture and sensory characteristics that are typical of mature cheese (Fox et al., 1993, Pollard et al., 2003). The traditional physical and chemical analysis methods have some limitations due to the fact that they are expensive and labour-intensive, so food processing industry is interested in looking for major compositional and quality traits to be determined online and in real-time. This seems to be an industrial need but can also be an opportunity for the characterisation and consequently for the valorisation of local cheeses produced by small dairies. Also, the interest of consumers is increasingly oriented towards traits that may have an impact on human health, such as the fat composition (González-Martín et al., 2020). In this context, chemical quality analyses are often out of reach for most of small local dairies, especially for the more expensive features, such as fatty acid composition. Local cheese productions generally show a high variability of characteristics, attributable also to process controls, which are often scarce and consist in not well standardised measures. Furthermore, small Italian dairy producers often directly transform milk obtained from their own animals (also of different species) into cheese productions. In this context, Erborinato, Tomino and Stracchino, examples of largely diffused cheeses in the Italian market, are usually produced from

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cow milk but also can derive from sheep and goat milk. Another type, called Marzolino, represents an historical cheese described in the XV century by Pantaleone da Confienza (1477) in his book "Summa Lacticiniorum" and as one of the most important Italian cheeses. The dairies of the Tuscan farms are generally characterised by modest size, modest technological level and non-specific equipment for each type of cheese. The use of low-cost techniques, such as near-infrared spectroscopy (NIRS), could help these small dairies to improve the process control and consequently the quality of cheese production. The increasing interest on the development of instrumental techniques is due to more objective, faster and cheaper assessments of cheese quality. Ideally, such techniques are also non-invasive and non-destructive. Near-infrared spectroscopy has demonstrated to be a reliable technique to predict compositional and quality characteristics of a variety of foodstuffs (Ozen & Mauer, 2002) and shows considerable advantages respect to the traditional analysis technique in terms of sample handling simplicity and preparation prior to spectral acquisition. In particular for cheese, NIRS has been used to determine several traits such as moisture (Wehling & Pierce, 1988), total solids (Rodriguez–Otero et al., 1995), pH, fat content, nitrogen fractions, volatile fatty acids, organic acids (Karoui et al., 2006) and fatty acids (Lucas et al., 2008a). For milk and cheese products, mid-infrared wavelength and fluorescence spectroscopy were often successfully applied to measure quality traits (Mazerolles et al., 2001, Karoui & Dufour, 2003). Nevertheless, Holroyd (2013) in a review suggested that cheese is the most difficult dairy product to analyse using NIRS, due to the heterogeneous characteristics and the wide variability of cheese types. In a recent review (De Marchi et al., 2018), several studies considered the application of the infrared technologies as NIRS and mid-infrared spectroscopy to predict the chemical composition of cheese and milk products, and to authenticate or discriminate dairy products. Furthermore, few studies have investigated the use of NIRS as a tool to predict fatty acid composition of cheese: Manuelian et al. (2017) studied the minerals content and the fatty acid profile of European commercial cheeses using transmittance spectroscopy, and González-Martín (2017) worked on cheeses using ewe, goat and cow milk mixed with different percentages. Most of the cited studies on NIRS application to assess cheese quality have focused on standardised commercial cheese, on few quality traits or a single sensing technique (visible, reflectance, absorbance). Differences among studies on dairy products were also relative to sample preparation: Cuibus et al. (2014) and Kraggerud et al. (2014) used mid-infrared spectroscopy on minced cheese samples, while in NIRS studies both intact (González-Martín et al., 2017) and grounded samples were used (Manuelian et al., 2017). Nevertheless, the milling treatment on one hand lengthens the time of the analysis, and on the other hand it allows an improvement of the predictive ability of NIRS (De Marchi et al., 2018).

The present study aimed to evaluate the performances of Fourier transform NIRS (FT-NIRS) to determine the chemical and the fatty acid composition of different types of cheeses from sheep and goat raw milk of small local dairies.

2 Material and Methods

This study was part of the Rural Development Program of Tuscany Region and considered a total of 95 cheeses produced by small local dairies located in the Province of Siena (Tuscany). Four types of cheeses produced with raw milk were collected: Marzolino (n=14), Erborinato (n=48), Tomino (n=19) and Stracchino (n=14). Marzolino, exclusively obtained from sheep milk, is a Tuscan traditional hard cheese, produced using vegetable rennet from artichoke plant and seasoned for 180 days. Erborinato is a soft or semi-hard cheese produced using a mix of goat and sheep milk, inoculated with *Penicillum* and seasoned for 90 days. Tomino and Stracchino are fresh soft cheeses obtained from sheep milk. For each type of cheese, samples were collected at the end of their own preparation process. Spectra were collected using FT-NIRS Antaris II model (Thermo Fisher Scientific, Waltham, MA, USA). Whole slices of cheese were exposed by a circular quartz cup spinner (60 mm of diameter) to an electro-magnetic scan in the absorbance mode. Each spectrum was the average of 32 scans collected during the automatic rotation of the cup from 10,000 to 4,000 cm^{-1} (1,000 to 2,500 nm) every 4 cm^{-1} and corrected against the background spectra of room environment. Absorbance data were collected as log(1/R), where R is reflectance, as it reproduces a more linear response in function of the concentration of the investigated trait (Birth & Hecht, 1987).

Samples were submitted to chemical analyses according to AOAC methods (AOAC, 2019): (i) moisture by freeze-drying to a constant weight; (ii) fat as ether extract; (iii) protein using Kjeldahl method; and (iv) ash in a muffle at 550°C. Lipids were extracted applying Folch modified method (Folch et al., 1957). Subsequently, fatty acid profile was determined according to Morrison & Smith (1964). Fatty acid methyl esters were prepared through esterification and analysed through gas

chromatography, using a Varian 430 apparatus (Varian Inc., Palo Alto, CA, USA) equipped with a flame ionization detector. Fatty acids separation occurred in a Supelco Omegawax TM 320 capillary column (Supelco, Bellafonte, PA, USA) and their quantification was performed through calibration curves, using nonadecanoic acid (C19:0) as internal standard. Fatty acids were expressed as total percentage and were identified and grouped as follows: saturated fatty acids (SFA), as the sum of C4:0, C6:0, C8:0, C10:0, C11:0, C12:0, C13:0, C14:0, C15:0, C16:0, C17:0, C18:0, C20:0, C21:0, and C22:0; monounsaturated fatty acids (MUFA), as the sum of C10:1, C12:1, C14:1, C16:1, C17:1, C18:1, C20:1, and C22:1; and polyunsaturated fatty acids (PUFA), as the sum of C18:2, C18:3, and C20:4 n6. All spectra were manipulated and processed through chemometric analysis using Unscrambler CAMO® software, and calibrations assays were performed by partial least squares (PLS) regression. To optimise the accuracy of calibration, several mathematical pre-treatments were applied: multiplicative scatter correction, standard normal variate with or without de-trending option and baseline correction. Outliers were identified and removed when necessary.

All the calibrations were performed using the full near-infrared spectra region and for each trait all models were evaluated, but only the best was reported. A full cross-validation using the 'leave-one-out' method was applied both to establish the number of latent variables in the regression model to avoid over-fitting and under-fitting (Faber & Rajko, 2007), and to validate the calibration model. The ability of the regression model was evaluated by calculating the following fitting statistics: the coefficient of determination (R^2) in calibration and in cross-validation (R^2cv), the root mean square error in calibration (RMSE) and in cross-validation (RMSECV), and the standard error in cross-validation (SECV). Goodness and accuracy of models were tested using residual prediction deviation (RPD) which is an index calculated as the ratio between the standard deviation of the set of samples and the SECV.

3 Results and Discussion

Average concentration, range and standard deviation of the proximate analysis and fatty acid profile of the different types of cheese are displayed in Table 1 (see last 2 pages). Only fatty acid groups and individual fatty acids most commonly analysed in cheese are reported in the tables (González-Martín et al., 2020). The average values for chemical composition were in line with those reported for other similar types of cheeses obtained through industrial process (Salvadori del Prato, 2001). Erborinato and Marzolino cheeses showed high variability for moisture, protein and fat, while Stracchino was the less variable product (Table 1). The greater variability observed for Erborinato cheese could be attributable to the characteristics of milk, as the farm used a mix of sheep and goat milk to produce this kind of cheese. Nevertheless, other factors such as specific cheese making techniques and absence of standardised production control protocols, within each farm, might have had an impact on the level of variability. Regarding the fatty acid profile, SFA was the most represented fraction in all cheeses, constituting about 2/3 of the total fatty acid profile (Table 1). The C14:0 and C18:0 fatty acids showed the highest concentration. González-Martín et al. (2020) suggested that the above mentioned SFA, together with C10:0 and C16:0 are the more abundant fatty acids in cheese. Monounsatured fatty acids ranged from 27 to 29%, which are typical values for milk fat, as suggested by Markiewicz-Kęszycka et al. (2013). Within this group, oleic acid (C18:1) showed the highest concentration, representing the most important fatty acid in milk produced by ruminant species (Strzałkowska et al., 2009). The percentage of PUFA varied from 4.2 to 5.6% of total fatty acids and C18:2 was the most present (from 3.1 to 4.2%) in all types of cheeses (Table 1). However, according to Nudda et al. (2005), the fatty acids content of cheese is strictly dependent on raw milk original profile and does not undergo change during the transformation process.

The results of the best FT-NIRS calibration models for each trait and the different mathematical treatments are reported in Table 2 (see last page). As regard to the mathematical pre-treatments on spectra, the best results were observed using multiplicative scatter correction and standard normal variate treatments for all features, similarly to Manuelian et al. (2017) who worked on different types of European cheeses. In addition, de-trending pre-treatment on spectra data set has been usually selected in order to correct the scatter effects in the spectra as reported by Lucas et al. (2008b) who studied fatty acid composition of cheese obtained from cow and goat milk. For all the traits, PLS required a high number of latent variables to obtain stable models; this is in accordance with Lucas et al. (2008b), whereas Manuelian et al. (2017) obtained better results using a lower number of latent variables in their models. Considering the proximate analysis, the R^2 ranged from 0.79 for protein to 0.93 for moisture. The best models in terms of R^2cv were obtained for moisture while ash, protein and fat showed values from 0.71 to 0.77 (Table 2). Results obtained by Stocco et al. (2019) on the freshly

cut cheese surface and concerning a whole spectrum of different cheeses, achieved R^2 comparable to our results for moisture and protein even if our errors in cross-validation were slightly higher probably due to the lower number of samples. Ash and lipid R^2 of our study were better than those reported by Stocco et al. (2019), even if those authors showed better results when the region from 350 to 1,830 nm, namely visible area in reflectance mode, was considered. Considering the near infrared region, Karoui et al. (2006) obtained higher R^2cv (0.94 and 0.86) both for lipids and protein but respect to our study, their study concerned only a single type of product (Emmental cheese) manufactured by different farms. Regarding the fatty acid groups, the best models in terms of R^2 were those for SFA and MUFA both in calibration and in cross-validation. The R^2 and R^2cv of PUFA were 0.77 and 0.67, respectively. Among individual fatty acids, the best results in calibration (R^2>0.75) were observed for C18:1 and some medium chain SFA, namely C8:0, C10:0, C12:0 and C14:0, even if the R^2cv were lower. The other C18 fatty acids (C18:0, C18:2, C18:3) performed worse than C18:1. As expected, RMSECV of all the traits tended to be slightly higher than the corresponding values in calibration (RMSE).

To our knowledge, few studies have investigated the use of FT-NIRS as a tool to predict fatty acid composition of cheese. Compared to the results obtained in the present study, Manuelian et al. (2017) reported higher R^2cv for most considered fatty acids in 19 varieties of European cheeses scanned as grounded samples using near infrared spectroscopy in transmittance mode. Nevertheless, those authors suggested the use of absolute concentration to quantify fatty acids in cheese (g/100 g cheese). Manuelian et al. (2017) reported that inadequate results were obtained when the spectra information was correlated with the percentage of total identified fatty acids. Lucas et al. (2008b), working on more than 400 samples of cow and goat fresh cheese in the visible/near-infrared region, reported higher R^2 for fatty acids groups compared with results obtained in the current study, but with higher errors evaluated in terms of SECV, whereas the R^2cv was slightly higher even if without a specific trend compared to our results. Nevertheless, those authors considered a fresh grated and crushed slice of freeze-dried cheese and it is known that the samples mincing improves the model performance. The model performance can be considered good enough for a rough screening in cheese if RPD is higher than 2 (Williams, 2014). Our study reported RPD values higher than 2 for moisture, protein and ash, whereas fat and fatty acids showed values between 1.5 and 1.9, except for C18:0, C18:3, and PUFA which exhibited RPD <1.5. González-Martín et al. (2020) reported lower RPD values for SFA, C8:0 and C14:0, higher RPD for C18:0 and C18:1, and values similar to those reported in the present study for the other fatty acids. On the other hand, Manuelian et al. (2017) reported more promising RPD for all the analysed traits. Nevertheless, all the cited studies used a larger sample size compared with our trial and it is known that increasing the number of samples in NIRS analysis can affect the goodness and the accuracy of the models. Esbensen et al. (2014) and Williams (2014) reported that some models with RPD higher than 3 are difficult to obtain due mainly to the data structure, in particular high or low concentration of only one sample. In addition, other factors such difficulty of sample preparation or incoherence with the reference test, the inaccuracy of the reference analysis and the complication of the instrument can affect the RPD results. Furthermore, Holroyd (2013) pointed out that RPD values of animal feed or meat are typically higher than values of milk products. Lastly, results of the present study, especially for fatty acid composition, could be improved following at least three different approaches: i) working on the main absorption for fat identification as suggested by Lucas et al. (2008b); ii) studying the spectra in terms of greater variable region as suggested by Karoui et al. (2006); iii) working on the sample presentation because different sample preparations between the infrared spectroscopy and the reference analysis can affect the model result as reported by De Marchi et al. (2018).

4 Conclusions

The analytical values obtained through reference methods in the present study showed high variability, likely due to the reduced control procedures of cheese making processes linked to the small local dairies from which products were obtained. However, results were in the same range of those reported in literature. The best calibration models were obtained using multiplicative scatter correction and standard normal variate spectra pre-processing methods. Calibration models showed adequate R^2 and R^2cv for proximate analysis (moisture, protein, fat and ash), fatty acid groups (SFA, MUFA and PUFA) and some individual fatty acids (C8:0, C10:0, C12:0, C14:0 and C18:1). The RPD values were higher than 2 only for moisture, protein and ash content, and were lower than those observed by other authors working on standardised industrial cheeses. This suggests that FT-NIRS models could be used only to determine moisture, protein and ash content, while further implementation is needed for the other traits before using the developed models as a routine analysis

method. Lastly an increase of the sample size for each type of cheese could be a key factor to improve both the goodness and the accuracy of calibration models. The availability of accurate calibration models could allow the use FT-NIRS technology to small dairies as a reliable and economic method to routinely assess the quality of cheese.

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Table 1 Descriptive statistics of chemical composition and fatty acid profile (%) obtained by reference analyses of cheese samples

	Erborinato (n=48)				Marzolino (n=14)				Stracchino (n=14)				Tomino (n=19)			
Trait	Mean	SD	Minimum	Maximum	Mean	SD	Minimum	Maximum	Mean	SD	Minimum	Maximum	Mean	SD	Minimum	Maximum
Moisture	38.22	5.73	20.55	48.58	30.73	6.50	25.15	51.52	50.56	4.59	40.53	56.82	61.75	4.85	51.76	68.83
Protein	28.79	6.05	17.39	38.85	30.16	4.38	19.33	38.43	19.78	2.28	17.36	24.60	16.90	3.21	12.59	22.63
Fat	29.25	5.46	19.60	40.29	33.82	4.74	25.94	41.17	26.78	2.53	22.78	31.42	19.77	5.61	10.10	28.19
Ash	3.72	1.19	2.17	6.60	5.27	0.94	2.70	6.07	2.83	0.46	1.95	3.66	1.58	0.57	0.79	2.77
C8:0	1.88	0.66	0.56	2.67	1.73	0.70	0.48	2.32	1.86	0.48	1.13	2.56	1.67	0.64	0.71	2.74
C10:0	7.02	2.68	1.99	10.15	5.83	2.34	1.90	8.01	6.06	1.64	3.85	8.84	5.69	2.51	2.44	10.80
C12:0	3.99	1.15	1.62	5.61	3.51	1.12	1.64	4.61	3.60	0.73	2.59	4.79	3.51	1.20	1.98	6.60
C14:0	10.95	1.42	8.09	13.19	10.49	1.42	7.87	11.79	10.60	0.94	8.91	12.08	10.94	1.38	9.19	13.45
C18:0	10.63	2.1	7.38	15.80	10.64	1.30	8.87	12.88	11.22	2.11	9.00	15.42	10.64	1.71	7.61	14.22
C18:1	25.68	4.03	20.51	15.80	27.8	4.08	23.85	35.58	27.32	2.84	22.22	31.37	26.19	4.73	18.33	32.68
C18:2	3.15	0.67	1.85	4.30	4.23	0.49	2.87	4.71	3.70	0.61	2.65	4.40	3.41	0.53	2.47	4.39
C18:3	0.95	0.21	0.36	1.53	1.23	0.32	0.93	1.75	1.08	0.33	0.58	1.63	0.96	0.32	0.27	1.32
SFA	68.79	4.46	58.65	74.67	65.46	4.52	56.51	69.43	66.59	2.93	61.76	71.93	67.88	5.25	60.46	76.80
MUFA	27.01	4.01	22.34	35.43	28.96	4.11	24.85	36.88	28.51	2.76	23.26	32.62	27.61	4.69	19.68	34.72
PUFA	4.21	0.74	2.78	5.92	5.58	0.66	4.08	6.61	4.90	0.87	3.49	6.13	4.51	0.80	3.11	5.62

SD - standard deviation; SFA - saturated fatty acids; MUFA - monounsaturated fatty acids; PUFA - polyunsaturated fatty acids

Table 2 Calibration and cross-validation results of chemical composition and fatty acid profile in intact slice of cheese samples

Trait	n	LV	Mean	SD	R^2	RMSE	R^2cv	RMSECV	SECV	RPD	MT
Moisture	95	11	43.90	11.86	0.93	3.11	0.89	3.96	3.96	3.0	1
Protein	90	7	25.13	7.20	0.79	2.29	0.74	2.77	2.78	2.6	2
Fat	95	15	27.55	6.55	0.84	2.61	0.71	4.19	4.46	1.5	1,3
Ash	95	13	3.42	1.49	0.88	0.51	0.77	0.72	0.72	2.1	2,3
C8:0	95	15	1.81	0.61	0.77	0.30	0.61	0.40	0.40	1.6	1,4
C10:0	95	15	6.38	2.43	0.80	1.11	0.63	1.52	1.53	1.6	1
C12:0	95	15	3.77	1.05	0.82	0.46	0.66	0.65	0.66	1.7	1
C14:0	95	15	10.84	1.31	0.76	0.65	0.59	0.85	0.86	1.6	1,3
C18:0	95	15	10.79	2.02	0.46	1.39	0.26	1.77	1.77	1.1	1
C18:1	95	15	26.25	3.93	0.86	1.52	0.71	2.20	2.22	1.8	1,3
C18:2	95	15	3.42	0.69	0.71	0.38	0.57	0.46	0.47	1.5	2,3
C18:3	95	15	0.99	0.27	0.57	0.28	0.40	0.22	0.22	1.3	1
SFA	87	15	67.92	4.33	0.89	1.53	0.75	2.35	2.40	1.9	1,3
MUFA	89	15	27.56	3.87	0.88	1.39	0.74	2.08	2.10	1.9	1,3
PUFA	95	15	4.53	0.85	0.77	0.40	0.67	0.58	0.63	1.4	1,3

LV - number of latent variables; SD - standard deviation; R^2 - coefficient of determination in calibration; RMSE - root mean square error of calibration; R^2cv - coefficient of determination in cross-validation; RMSECV - root mean square error of cross-validation; SECV - standard error in cross-validation; RPD: residual prediction deviation in cross-validation; MT - mathematical treatment (1. multiplicative scatter correction, 2. standard normal variate, 3. de-trending, 4. baseline correction); SFA – saturated fatty acids; MUFA – monounsaturated fatty acids; PUFA – polyunsaturated fatty acids