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Abstract

Milk samples from 1,264 cows in 85 farms were authenticated for different farming-systems using a 10-fold cross-validated linear-discriminant-analysis using Fourier-transform infrared spectra (**FTIRS**) and gas-chromatographic fatty-acid (**FA**) profiles. FTIRS gave correct classification greater than FAs (97.4% *vs.* 81.1%) during calibration, but slightly worse in validation (73.5% vs 77.3%) and their combination improved the results. All milk samples were processed into ripened model-cheeses, and analyzed by near-infrared-spectrometry (**NIRS**), by proton-transfer-reaction time-of-flight mass-spectrometry for their volatile organic compound (**VOCs**) fingerprint and by panel sensory profiling (**SENS**). Farming-system authentication on cheese samples was less efficient than on milk, but still possible. The instrumental methods yielded similar validation results, better than SENS, and their combination improved the correct classification rate. The efficiency of the different technics was affected by specific farming systems. In conclusion, dairy products could be discriminated for farming-systems with acceptable accuracy, but the methods tested differ in sampling procedure, rapidity and costs.

Key-words: food origin discrimination; food authentication; food quality monitoring, volatile organic compound; silage feeding.

1. Introduction

Consumers now demand greater transparency concerning the origin of their foods. As an example, in the case of dairy products this includes access to information on the diets of cows and the systems in which animals are farmed (Abbas et al., 2018; Cossignani et al., 2019). The authentication of food has considerable importance (Valdes et al., 2018; Esteki et al., 2018; Medina et al., 2019), especially when it comes to labeled products, as the raw materials used in manufacturing them have to conform to regulatory specifications. Certain farming practices have a significant effect on the quality of milk and dairy products (Martin et al., 2005; Bovolenta et al., 2014; Bergamaschi et al., 2015). In recent years, several sources of information have been used to discriminate between foods obtained from different farming systems. For example, Coppa et al. (2015) showed that the fatty acid (**FA**) profile can be used to discriminate milk from fresh forage feeding systems. Plant secondary metabolites, such as terpenes and carotenoids, have been used to discriminate between pasture-derived and cereal-derived milk and cheese (Slots et al., 2009; Tornambé et al., 2006). However, the traditional reference methods for analyzing milk and cheese components to provide information useful for farming system traceability, e.g. gas chromatography (**GC**; Capuano et al., 2014; Coppa et al., 2015) and sensory analyses (Bérodier et al., 1997; Martin et al., 2005) are expensive and time consuming, require highly skilled operators, and are not easily adapted to online monitoring and routine analysis on a large scale. Hence, the new challenge is to develop rapid, low-cost screening techniques able to authenticate food products with characteristics that meet consumer expectations. Fourier transform infrared (**FTIR**) spectroscopy, near infrared (**NIR**) spectroscopy, and proton-transfer-reaction time-of-flight mass spectrometry (**PTR-ToF-MS**) for analyzing, respectively, liquids (milk), solids (cheese) and volatile organic compounds (**VOCs**) are acknowledged tools for meeting this challenge. These techniques are characterized by high throughput and the ability to rapidly collect a large amount of information that can be used to fingerprint many food samples. Needing no sample preparation, they are also non-destructive, simple, and rapid. As an example, in the dairy industry, specific FTIR calibrations have been developed for monitoring many of the chemical and technological characteristics of milk (ICAR, 2012; Bittante, Penasa, & Cecchinato, 2012). Recently, NIR has been used also to discriminate some cheese varieties (Lucas, Andueza, Ferlay, & Martin, 2008). PTR-ToF-MS has been used to determine the volatile fingerprint of a wide number of model cheeses in order to study individual phenotypic (stage of lactation, parity, and milk yield) and genetic factors (Bergamaschi et al., 2015 and 2016a).

The ability to discriminate food products of animal origin depends on several sources of variation, such as the animals' genetics, herd management, farming system, food manufacturing process, and ripening conditions. So far, no-one has compared the different sources of information available for authenticating milk and cheese derived from different farming systems on a large scale and on the same food samples and using the same statistical method. Moreover, validation has not always been carried out on samples different from those used to define the discriminant analysis functions.

The aim of the present study was to compare the effectiveness of different sources of information for discriminating foods origin in relation to the farming system of production using milk and cheese as a case study. We compared the sources of information most commonly used to distinguish milk (FTIR spectra, and fatty acid profiles by GC) and cheeses (NIR spectra, flavor fingerprinting by PTR-ToF-MS, and sensory description by a trained panel) according to farming system. Our specific objectives were: a) to compare all 5 sources of information using a large number of milk and cheese samples from several farms; b) to use cheeses to compare the 3 latter sources of information produced from the same milk samples used to compare the two former sources of information; c) to standardize all the sampling, processing and analyzing procedures in order to minimize potentially confounding sources; and d) to adopt the same statistical method, based on cross-validation, to analyze all sources of information.

2. Material and methods

2.1. Experimental setup

The present study is part of the Cowability-Cowplus project widely described in Bittante et al. (2015) and Stocco et al. (2017). Briefly, we sampled a total of 1,264 Brown Swiss cows from 85 herds located in Trento Province (northeastern Italian Alps). The farming systems were classified into 5 groups: the first two groups comprised cows reared in traditional Alpine dairy systems (tied cows milked at stalls, fed mainly hay and some compound feed), but differing according to whether or not automatic feeders (**AF**) were used to distribute the compound feed at the stall; the third comprised animals kept in modern dairy systems (loose-housed in larger, modern facilities with milking parlors, and with feedstuffs distributed separately in the mangers and without the use of total mixed rations (**TMR**). The fourth and fifth dairy systems were modern farms using TMR, without or with corn silage, respectively. In order to devise appropriate strategies for authenticating dairy systems, we also merged the 5 original groups into 3. The two groups of traditional systems (with and without AF) were pooled into one, and the modern dairy systems were reclassified according to whether or not the diets included silages (the modern group using hay and compound feed was pooled with that using TMR without silage). The individual cows presented different numbers of lactation (1 to 5), days in milk (5 to 449), and daily milk yields (24.3±7.9 kg×d^{-1}) (Table 1).

2.2. Milk sampling

On a given day, only 1 herd (generally 15 cows per herd) was sampled during the evening milking. Two milk subsamples were taken from each cow and immediately refrigerated at 4 °C without any preservative. One subsample (50 mL) was taken to the milk quality laboratory of the Breeders Federation of the Province of Trento (Trento, Italy) for chemical composition analysis. The other subsample (2,000 mL) was taken to the cheese-making laboratory of the Department of Agronomy, Food, Natural Resources, Animals and Environment of the University of Padova (Legnaro, Italy); an aliquot (1,500 mL) of this subsample was used for cheese production, and the remainder was analyzed for its fatty acid profile. All samples were processed for analysis and model cheese manufacture within 20 h of collection (Cecchinato and Bittante, 2016).

As this study summarizes the results of a large research project and compares 5 different sources of information that have been used for characterizing the milk and cheese samples in relation to farming systems, animal's genetics and health, environmental impact, etc., it is not possible to describe in great detail each of the analytical method used, but for each one the most important issues are presented and a specific reference article, easily accessible, with all the details is cited.

2.3. FTIR analysis

All individual milk samples were analyzed by Fourier-transform infrared spectroscopy (MilkoScan FT6000, Foss, Hillerød, Denmark) over the spectral range from wavenumber 5,011 to 925 cm^{-1}, corresponding to wavelengths 2,000 to 10,800 nm, yielding a total of 1,060 waves (Table 2), according to the procedure described by Ferragina et al. (2015). Spectra were stored as absorbances (A) using the transformation A = log(1/T), where T is the transmittance. Before data analysis, 10 replicate spectra obtained from 2 aliquots averaged to obtain one spectrum for each milk sample.

2.4. Gas chromatography analysis

Fatty acid analysis was performed as described in detail by Mele et al. (2016). Briefly, each milk sample was centrifuged at 5,000 × g for 30 min at 4 °C. Thirty milligrams of fat were collected in a vial, and mixed with 3 mL of hexane and 0.3 mL of 2 M methanolic solution of KOH. The mixture was incubated for 5 min at room temperature after the addition of 0.25 mg of $NaHSO_3 \times H_2O$. The samples were then centrifuged at 3,000 × g for 3 min at 4 °C, and the upper layer was collected for GC analysis. Fatty acid composition was determined using a ThermoQuest gas chromatograph (ThermoElectron Corp., Waltham, MA, USA) equipped with a flame-ionization detector and a high polar fused-silica capillary column (Chrompack CP-Sil88 Varian, Middelburg, the Netherlands; 100 m, 0.25 mm i.d.; film thickness 0.20 μm). The carrier gas was helium at a flow rate of 1 mL/min. A split/splitless injector was used. A sub-sample was injected under the following GC program: the initial oven temperature (60 °C) was held for 1 min, then increased to 173 °C at a rate of 2 °C/min and held for 30 min, increased to 185 °C at 1 °C/min and held for 5 min, and finally increased to 220 °C at a rate of 3°C/min and held for 19 min. The injector temperature was set to 270 °C, and the detector temperature was set to 300 °C. Individual FAME were identified by comparison with a standard mixture (52 Component FAME Mix, GLC-674; Nu-Chek Prep Inc., Elysian, MN, USA). The isomers of C18:1 were identified with reference to commercial pure standards (47199, 46903, 46905; Supelco, Bellefonte, PA). A butter reference standard (BCR 164; Commission of the European Communities, Community Bureau of Reference, Brussels, Belgium) was used to estimate correction factors for the short-chain fatty acids, as previously described by Mele et al. (2016). A total of 47 fatty acids were identified in each milk sample and expressed as grams per 100 g of the total fatty acid content.

2.5. Model cheese making

An individual model cheese was produced from the milk of every cow sampled according to the cheese-making procedure described by Cipolat-Gotet et al. (2013). Briefly, 1,500 mL of raw milk from each cow was heated to 35 °C in a stainless-steel micro vat, and a formulation of thermophilic starter culture was added (Delvo-Tec TS-10A DSL; DSM Food Specialties, Delft, the Netherlands). A commercial rennet (Hansen standard 160, with 80 ± 5% chymosin and 20 ± 5% pepsin; 160 international milk clotting units/mL, Pacovis Amrein AG, Bern, Switzerland) was then added, and the resulting curd from each vat was cut, drained, shaped into wheels, pressed, salted, and weighed. The model cheeses were ripened for 60 d then analyzed for chemical composition.

2.6. NIR analysis

Cheese samples were placed in a 100 mm diameter ring cup. This NIR instrument (FoodScan, Foss Electric A/S, Hillerød, Denmark) operates in transmittance mode with a moving monochromator (Table 2) scanning the region from wavelength 850 to 1,048 nm (corresponding to wavenumbers 11,764 to 9,524 × cm^{-1}) with data points at intervals of 2 nm, giving a total of 100 waves. From every cheese sample, 16 replicated entire spectra were acquired and averaged before statistical analysis.

2.7. PTR-ToF-MS analysis

Volatile organic compound analysis was performed as described by Bergamaschi et al. (2015 and 2016). Briefly, 3 g of each cheese sample, hitherto stored at -80 °C, were thawed at 20 °C for 6 h then placed in glass vials (20 ml, Supelco, Bellefonte, USA), capped with PTFE/Silicone septa (Supelco), then measured with a PTR-ToF-MS 8000 instrument (Ionicon Analytik GmbH, Innsbruck, Austria) (Table 2). The conditions in the drift tube of the PTR were as follows: temperature 110 °C, drift pressure 211 Pa, drift voltage 500 V. Internal calibration and spectrometric peak extraction were performed according to the procedures described by Cappellin et al. (2010, 2012), resulting in the identification of 619 spectrometric peaks per cheese sample. Headspace volatile organic compound concentrations, expressed as parts per billion by volume, were estimated using the method described by Lindinger, Hansel, & Jordan (1998).

2.8. Sensory profile

Sensory analysis was performed by a trained panel as described in detail by Cipolat-Gotet et al. (2018), while the reference standard and the protocol scorecard were in accordance with Bérodier et al. (1997) and Lavanchy et al. (1993). Briefly, 14 panelists (6 females and 8 males, age 35.6±11.8 years) were selected and trained in cheese evaluation under the direction of a panel leader. Their task was to assess the cheese samples according to 7 main sensory descriptors: intensity of smell, intensity of flavor, intensity of salt and sour tastes, elasticity, firmness, and moisture. These traits were ranked on a 13-point discontinuous scale (from 1 to 7, including half points). A further level of sensory description was introduced for smell and flavor: after assessing their overall intensities, the assessors had to evaluate on a 4-point discontinuous scale the intensities of 4 families of descriptors, each composed of several detailed attributes, giving a total of 20 traits for smell and 20 for flavor.

2.9. Statistical analysis

2.9.1. Data processing

The absorbance values of every wavelength in the FTIR spectra (1,060 waves) of the milk (1,222 samples), and in the NIR spectra (100 waves) of the cheese (915 samples) were centered and standardized to a null mean and a unit sample variance. Next, we calculated Mahalanobis distances using the standardized FTIR and NIR spectra data to detect outliers. Having decided from examining the plot to exclude only spectra with a very high probability of being outliers, we discarded those with a Mahalanobis distance greater than 3 times the standard deviation. To increase the normality of distribution of absorbance value and improve LDA, first-derivative Savitzky-Golay was also applied to the spectra as a mathematical pretreatment (Savitzky & Golay, 1964) for both FTIR spectra of milk and NIR spectra of cheese.

The 47 FAs were centered, and values greater than three times the standard deviation were discarded as outliers.

The 619 spectrometric peaks characterizing the volatile profile of each model cheese (1,075 samples) were standardized within each day of analysis (15 days) to equalize any data variability resulting from the effect of this environmental factor on the proton transfer reaction peaks, then analyzed according to the procedure described in detail by Bergamaschi et al (2015). Some highly-correlated peaks ($r > 0.95$; $P < 0.001$), corresponding to isotopes of the same volatile organic compounds, were removed from the dataset before the statistical analyses.

The sensory descriptors were edited according to the procedure described by Cipolat-Gotet et al. (2018). As each cheese sample was evaluated by several panelists, one record per cheese sample was obtained by analyzing the 6,612 scorecards using the SAS Mixed Model procedure (SAS Institute Inc., Cary, NC) with the 1,224 model cheeses/cows included in the statistical model as fixed effects, and the 14 panelists as random effects: the least square means of each cheese/cow were then extracted and used as independent observations for the multivariate analysis.

2.9.2. Linear discriminant analysis

The linear discriminant analysis (**LDA**) was used for testing the authentication of farming systems of origin of milk and cheese as it is one of the most frequently used methods adopted for food authentication (Granato et al., 2018a; Jiménez-Carvelo et al., 2019). Five LDAs, one per source of information, were carried out using the MASS package in R to determine which combination of variables contributed most to the differences in the milk and cheese samples from the various farming systems. Briefly, we categorized the farming systems into a group of 5 and a group of 3, as described above. A 10-fold cross-validation procedure was used to estimate the discrimination capability of the LDAs, to avoid overfitting, and from this we identified the minimum number of variables (waves, fatty acids, spectrometric peaks, or sensory descriptors) required to authenticate the various dairy systems. The data in each dataset were divided randomly into 2 sub-sets: a training set (approximately 75% of the data), which was used to calibrate the model, and a testing set comprising the remaining data (25%), which was used for cross-validation. This process was repeated 10 times, using different sub-sets each time. Two additional LDAs were carried out on the combined instrumental information from the analyses of milk (FTIRS + FAs) and of cheese (NIRS + VOCs), considering only those samples for which both types of data were available.

2.9.3. Comparison of discriminant ability of LDA models by logistic regression

The discriminating ability of a model is usually evaluated through a graphical representation of data of each model. In this case, the large number of models tested (5 sources of information + 2 combination × 2 number of farming systems to be discriminated = 14 LD analyses), the large number of samples analyzed (915 to 1,124 per source) and the possibility to plot only two latent variables make unfeasible this approach. To test the differences in the discrimination ability of the 14 models compared here considering the specific distribution and variability of each source of information, a logistic regression was carried out. Correct classification rates for all the LDA models were coded as binary variables (0, 1), where 1 indicated correct classification of the milk or cheese sample. This new variable was analyzed by logistic regression using the SAS LOGISTIC procedure (SAS Institute, 2012) according to the following model:

$$\mathrm{logit}(\pi)=\log\left(\frac{\pi}{1-\pi}\right)-\alpha+\beta' x_1$$

where $\pi = \Pr(Y = 1|x)$, which is the response probability (odds ratio) of correct classification; α is the intercept of the parameter; $\beta = (\beta_1,\ldots, \beta_i)$, which is the vector of the i slope parameters; and x is a vector for the fixed effects of the methods: source of information (7 levels), dairy system (3 or 5 levels), and the source-dairy system interaction (21 or 35 levels). The odds ratio estimates together with their confidence intervals for each source of information were used to plot these across the different dairy systems (Figure 1 a and b). For a better understanding of the nature of the information available through the infrared spectra, a principal component analysis was carried out on the milk FTIR spectra and on cheese NIR spectra.

3. Results and discussion

Only few published papers compared different instruments with the same pool of samples, and none compared, as the present study does, several sources of information on a large scale to discriminate milk and cheese according to the farming system in which cows were reared. Scampicchio et al. (2016) studied the possibility of discriminating 189 milk samples using 4 sources of information and their combination: chemical components of milk predicted from FTIR spectra (they did not used the absorbance values for discrimination); NIR absorbance spectra; FA profiles from GC; and stable isotopes ($^{13}C/^{12}C$ and $^{15}N/^{14}N$). They aimed at discriminating milk samples according to region (40 samples from North Tyrol, 130 from South Tyrol and 19 from other origin "Europa"), season (71 spring, 42 summer, 14 autumn, 43 winter, and 19 unknown) and heat treatment (90 raw milk, 77 HTST, and 19 UHT). Using a principal component analysis, they obtained very poor results whether using each one of the four sources of information for discriminating region, season or treatment. It worth noting that the distribution of sample was strongly unbalanced because samples from “Europa” were all and the only ones UHT treated and with unknown season of production. From North Tyrol, only raw milk was sampled whereas from South Tyrol both raw and HTST milk was sampled. Using PLS-DA discriminant analysis the incidence of samples not assigned to the region of production was 24%, 6%, 5%, and 18%, and the correct classification of assigned samples was 64%, 93%, 92% and 87% using stable isotopes, milk composition predicted by FTIRS, fatty acid profile, and NIRS spectra, respectively. Combining all the sources of information the unassigned samples were 4% and correct classification of assigned was 96%. These values are similar to ours, but were all obtained from a training (calibration) dataset and no testing (validation) results were available, so that it is not possible to evaluate the rate of over-fitting of discrimination (Granato et al., 2018a).

3.1. FTIR spectra of milk vs. NIR spectra of cheese to discriminate dairy systems

The discrimination abilities of the two infrared spectroscopy technologies we used to analyze milk before processing and the corresponding ripened cheeses were very different, especially with the training subsets, with milk always more efficient than cheese. When discriminating between the 3 farming systems, 97.4% of milk samples *vs.* 75.9% of cheese samples were correctly classified during training, and 73.5% *vs.* 67.1%, respectively, during testing (Table 3). When discriminating between the 5 farming systems, we obtained corresponding values of 98.6% *vs.* 66.8% during training, and 65.0% *vs.* 52.1% during testing (Table 3). When we looked at the discrimination abilities of specific farming systems, we obtained better results with milk than with cheese only for the traditional farming system and the modern system with silage when discriminating between the 3 farming systems (Table 4), whereas the results with milk were always better when discriminating between the 5 farming systems, with the only exception modern farms not using TMR (Table 5).

The underpinning mechanism responsible for fingerprinting and samples authentication by infrared spectroscopy rely on the chemical modification of milk produced in different dairy system and of the cheese obtained processing that milk.

As seen in a previous study on this same or other datasets, the fatty acid profile of milk is affected by dairy system (Mele et al., 2016), and could be used for dairy system authentication (as described in the next section). Moreover, fatty acid profile of milk can be predicted by infrared spectroscopy (Ferragina et al. 2015), and this is a base for expecting that infrared spectra of milk could be effective in dairy system authentication. But dairy system affects also other aspects of chemical composition of milk that could find a correlation on milk spectrum: detailed protein profile, amino acids, some minerals, some enzymes, etc. all these changes are causing derived modification on the composition of cheese. The differences among dairy systems found in the detailed volatile profile of cheese is a testimony (Bergamaschi et al. 2015). So, also the infrared spectrum of cheese is expected to reflect the effect of dairy system on some aspects of the cheese composition.

It is not possible to know whether the differences in terms of discriminating ability in favor of milk spectra arise from the characteristics of the dairy product analyzed or the type of infrared spectrometry used, given that different infrared spectrometers are used for analyzing liquid and solid materials. In our study, we compared the tools most commonly used for analyzing milk and cheese (González-Martín et al., 2011; Ferragina et al., 2015). For milk, this was a Fourier-transform infrared spectrometer (MilkoScan FT6000, Foss, Hillerød, Denmark) (Table 2), commonly used throughout the world in laboratories that analyze samples as part of milk recording schemes. This instrument can cover a very wide spectrum (more than 1,000 individual waves) ranging from part of the near-infrared region (NIR or SWIR), mid-infrared (MIR or MWIR) to part of the far-infrared region (FIR or LWIR), as discussed in a previous study (Bittante and Cecchinato, 2013). For cheese, we used a NIR spectroscopy system (Table 2), also manufactured by Foss specifically for analyzing samples of solid foods. This instrument operates over a narrow range (recording 100 waves) of the NIR region of the electromagnetic spectrum. The large superiority of information given by FTIRS than by NIRS during training is probably due, in particular, to the greater number of data points available for the calibration equations. This statistical advantage is, in large part, lost during testing, showing that a large number of data points is not strictly necessary and it can cause over-fitting during calibration.

It is worthwhile of noting that McQueen et al. (1995) compared NIR and MIR spectroscopic techniques (different from ours) to predict the protein, fat and moisture contents of cheese, and found prediction by NIR spectroscopy to be more precise.

Obviously, the differences may also be explained by the fact that we sampled the milk on the farm at the time of milking, and analyzed it within a few hours, during which time the sample was kept sealed and refrigerated. This means that it fully reflects the conditions of the different farming systems considered and the individual farms within system. The model cheeses were all made in the same laboratory following a standardized procedure (Cipolat-Gotet et al., 2013) with the aim of controlling all external sources of variation as much as possible. The cheeses were then sampled after 2 months of ripening, during which many physical, biochemical and microbiological changes had taken place (Fox et al., 2004). We then analyzed them to look after two months for residual effects of the differences in the raw materials from which they were manufactured. Interestingly for dairy industry, our results show that after 2 months of ripening the cheeses still exhibited the effects of farming system on the raw materials (milk) from which they were made (Table 3).

As supportive evidence based on multivariate analysis are available for milk fatty acid profile (Mele et. al., 2016) and volatile organic profile of cheese (Bergamaschi et al., 2015), but not for infrared spectra, the main results of a principal component analysis was carried out also on the milk FTIR spectra and on cheese NIR spectra and the results are available as supplementary material (Supplementary Figures S1 and S2). Principal component analysis is a useful instrument for understanding the relationships among many variables, like infrared absorbencies, to reduce the dimensionality of a large database, and also to visualize different groups of samples (O'Callaghan et al., 2017), but it is also criticized because it provides only a qualitative view of the data and it is not specific for discriminating analyses (Granato et al., 2018b).

3.2. GC fatty acid profile vs. FTIR spectra for discriminating milk samples

A summary of the overall results of the 10-fold cross-validated LDAs of the FTIR spectra and the FA profiles of milk are presented in Table 3. When discriminating between 3 farming systems, we found FTIRS to have a much higher average rate of correct classification in training than the FAs (97.4% *vs.* 81.1%). During testing, however, the rate of correct classification by FTIRS was much lower (73.5%), and with FAs almost unchanged (77.3%), so the odds ratio of the FAs was much lower than that of FTIRS in training, but higher in testing (in both cases the 95% confidence interval of the odds ratio did not include 1.00, the reference value attributed to FTIRS).

Combining both sources of information, we obtained a further increase in the percentage of milk samples correctly classified during training (99.6%), but the improvement was only marginal during cross-validation (77.9%) compared with FAs alone (Table 3).

Compared with the 3-system classification, when we tried to discriminate milk samples classified into 5 different farming systems, we found that the LDAs of the training subset resulted in lower percentages for FAs (70.0%), but not for FTIRS (98.6%) nor for FTIRS+FAs (99.8%). However, with the testing subset, we found that, overall, fewer milk samples were correctly classified with both methods individually (both at 65%), and also combined (70.3%).

The results of the LDAs for each individual farming system are shown in Table 4 (the 3-system classification) and Table 5 (the 5-system classification). More precisely, FTIRS spectra of the milk samples in the training datasets always resulted in very high rates of correct classification, whether discriminating the 3- or the 5-system classifications (95.8 to 99.7%). With the FAs the percentages of correct classification were lower and more variable (68.8 to 88.9%), and lower still for the two traditional farming systems (62.2% without AF, and only 37.9% with AF), which are not easily distinguishable from each other.

The results of the LDAs using FAs were slightly lower with the testing datasets than the training datasets for both the 3- and 5-dairy system classifications. In contrast, the FTIRS LDA yielded fewer correct classifications with the testing datasets than the training datasets, and it was often less efficient than the FA LDAs.

FTIR and GC analyses have previously been used to discriminate milk origin. For example, Capuano et al. (2014) combined FTIR spectra with chemometric techniques to develop a classification model for authenticating milk according to whether the cows were grass fed, pasture grazed, or organically farmed. Of the 116 tank milk samples they analyzed, an average of 80% were correctly classified in cross-validation. Some specific FAs, such as cyclopropane, have been identified as markers of milk from farms using maize silage (Caligiani, Marseglia, & Palla, 2014). There have also been reports (Ferlay et al., 2008; Hurtaud, Dutreuil, Coppa, Agabriel, & Martin, 2014) of a large effect of FA profile (especially odd- and branched-chain) when discriminating between milk from dairy systems using hay and fresh herbage and milk from systems using maize silage-based diets. The milk derived from systems using corn silage (50 bulk milk samples) had lower contents of polyunsaturated fatty acids than milk derived from systems using herbage (50 bulk milk samples), and was not misclassified by leave-one-out cross-validation (Hurtaud et al., 2014). Our rates of discrimination using fatty acids were similar to those of Coppa et al. (2015), who correctly classified 32 samples of bulk milk from cows fed diets with over 50% of the dry matter content constituted by maize silage. In our experiment, validation using milk FAs generally yielded slightly better rates of correct classification than validation using FTIR spectra. It should be evidenced that this small superiority probably does not compensate, at industry level, the major costs, complexity and time needed for GC analysis respect to infrared spectra acquisition. The dairy systems using fresh herbage (not tested in this study) are more easily distinguishable, as confirmed by Capuano et al. (2014), who reported that bovine milk FAs had greater sensitivity and specificity (about 100% in external validation) than milk FTIR spectra.

3.3. NIR spectra vs. volatile fingerprinting for discriminating cheese samples

The overall results of the 10-fold cross-validated LDAs using the NIR spectra and VOCs of cheese are given in Table 3. When discriminating between the 3 farming systems, we found the average correct classification rate in training to be greater with VOCs (83.1%) than NIRS (75.9%). The results of the cross-validation show that both techniques had lower rates of correct classification and were similar to each other (NIRS, 67.1%; VOCs, 66.9%). Combining the two sources of information (NIRS+VOCs) increased the levels of correct classification of the cheese samples during the training analysis (94.3%), and also during cross-validation, although to a lesser degree (71.5%) (Table 3). When we tried to discriminate the cheese samples from the 5 different farming systems, the results of the LDA of the training subset revealed lower percentages for NIRS (66.8%) and for VOCs (75.1%), but not for NIRS+VOCs (95.3%), compared with discrimination of cheese samples from the 3 farming systems. With regards to the testing subset, we observed a decrease in the overall rates of correct classification of cheese samples with both methods individually (52.1% for NIRS, 48.2% for VOCs), and also in combination (57.3%).

The results of the LDAs for discriminating each individual farming system from cheese characteristics are reported in Tables 4 and 5. In particular, the rate of correct classification of cheese samples based on VOCs and the training dataset was higher than that based on NIRS, for both the 3- and 5-dairy system classifications. The LDAs based on NIRS and VOCs yielded slightly better results with the training datasets than with the testing datasets, for all 3 or 5 farming systems.

It is well documented that infrared spectra reflect the chemical compositions of specific milk and cheese samples, and they have been utilized mainly for prediction purposes (Wojciechowski and Barbano, 2016; Margolies and Barbano, 2017). NIRS has also been used for discrimination purposes, mainly to distinguish between cheese samples derived from pasture-based *vs.* silage-based systems, and derived from hay *vs.* silage-based systems. Andueza, Agabriel, Constant, Lucas, & Martin, (2013) reported classification rates higher than 90% when discriminating Abondance (n = 92), Tomme de Savoie (n = 107) and Cantal cheeses (n = 109) obtained from the milk of cows fed at pasture or on preserved forage using a 4-fold cross-validated partial least square discriminant analysis. The slightly lower rate of correct classification of the samples in our study is clearly a consequence of not having included pasture-based systems. Support for this explanation comes from reports (Martin, et al., 2005; Valenti et al., 2013) of the difficulty of distinguishing between dairy products derived from cows fed on hay and from cows fed on maize silage using spectra and terpenes as variables. It could be interesting, at industry level, the fact that flavor profile could be used to discriminate cheeses in relation to the residual effect of the farming system of the milk used for cheese-making, even after two months of ripening. But it is also of interest the fact that VOCs profile is not superior to NIRS spectrum in this regard.

3.4. Sensory traits for discriminating cheese samples

The results of the 10-fold cross-validated LDA using the sensory traits (SENS) of cheese are reported in Table 3. Whether discriminating between the 3 or the 5 farming systems, we found the average rate of correct classification in training with SENS (89.1% and 89.7%, respectively) to be much greater than with NIRs and VOCs, but much lower with the combination NIRS+VOCs (94.0% for the 3-system group, 94.3% for the 5-system group). On the contrary, the results of the cross-validation showed that a smaller percentage of samples were correctly classified by SENS than by any of the instrumental sources of information, as clearly shown in Figure 1. With respect to the individual farming systems, the rates of correct classification of cheese samples by SENS with the training datasets were very similar for all the farming systems (Tables 4 and 5) and particularly low when discriminating cheeses derived from cows fed on silages during cross-validation (Table 4 and 5).

Using the same model cheeses as in this study, we recently found that those derived from farms using silage were perceived as having greater firmness and less moisture (Cipolat-Gotet et al., 2018). Other authors (e.g., Martin et al., 2005) also reported that cheese was influenced by the presence or absence of maize silage in the cows' diets. However, we also found a large effect of individual herd within dairy system on 47 sensory descriptors, which varied from just under 15% to 70%. The other large source of variation observed in our previous study was the panelists. Evidently, the large variation among different types of farm and among panelists precludes obtaining a high rate of discrimination according to dairy system using the sensory profiles of cheeses. So, this methodology could be interesting as a research tool, but not at level of the dairy industry.

3.5. Future application of food authentication

The results of the present study confirmed that several chemical methods can be used for authenticating the milk and dairy products in relation to the dairy system of origin. The efficiency of discriminating analyses is not so high to allow an official certification on individual samples, but these technics could be used for a preselection of samples to be further studied and/or for monitoring milk or cheese suppliers with time. This could be particularly useful for dairy products with some process certification, like the protected designation of origin (PDO) cheeses of the European Union, whose norms of production define not only the area of production but also the dairy system and cows' feeding regime in relation to the use or not of grazing, silages, hay, concentrates (Bertoni et al., 2005). Also, the authentication of milk, cheeses and other typical dairy products obtained during the summer grazing on the highlands Alpine pasture could benefit from these methods (Buchin et al., 1999; Coppa et al., 2011; Bergamaschi et al 2016b).

The methods used on fluid milk (FTIR spectrum and GC FA profile) could be used to control the bulk milk supplied to dairies by individual farmers or commercial traders or the packaged milk supplied to retailers' chains by different dairies. The use of FTIR spectra for dairy system authentication is much more practical and less expensive than GC FAs, and could be used for characterizing milk for many chemical composition traits (ISO-IDF (2013) and technological properties (Bittante et al., 2012). On the other hand, FAs could be used also for a better certification of nutritional properties of milk in relation to human health (Shingfield et al., 2013; Mele et al., 2016).

The instrumental methods used for cheese authentication (NIR spectrum and VOCs profile) have demonstrated that also after 2-month ripening, still some influence of dairy farming system of origin of milk could be captured. In this case the possible interest is at level of cheese factory for monitoring cheese batches obtained from milk of different suppliers, but also at level of different PDO consortia or retailer chains for comparing different cheese producers. If, also in this case, the use of NIR spectra is much more practical and less expensive (Andueza et al., 2018), the use of VOCs profile could offer other information and certification of cheese flavor and possibly replace the use of sensory description actually mandatory according to the norms of production of some PDO cheeses (Bittante et al., 2011a and b; Ojeda et al., 2015).

4. Conclusions

In this study, we compared different sources of information for discriminating different farming systems from a large number of individual milk samples and from the individual model cheeses obtained from processing these same milk samples according to a standardized procedure. Our findings with regards to discriminating different farming systems were that: a) the results from the training subsets (calibration) were often high, especially for those sources of information with many data points; b) the results from the testing subsets (validation) were lower, and not much influenced by the number of data points available; c) discrimination between a larger number (5) of farming systems tended to be less efficient than between fewer (3) farming systems; d) the information from instrumental techniques is more effective than that obtained from the sensory descriptors developed by trained panelists; e) the information from instrumental techniques for analyzing milk was more effective than that from techniques for analyzing cheese, although after two months of ripening the cheeses still showed the influence of farming system; f) fatty acid profiles tended to be more effective than infrared spectra for milk sample validation, and combining them further increases discrimination ability; g) infrared spectra and volatile fingerprints are equally effective for cheese sample validation, and combining them further increases discrimination ability.

In terms of cost, rapidity and simplicity of acquisition of the information, the infrared spectra (FTIR for milk and NIR for cheese) coupled with LDA have proven to be valuable instruments for adding information on the farming system in which the food is produced. This could be used for a rapid identification of food batches presenting some discrepancies with declared origin to be further investigated. The accuracy of discrimination could be further improved combining them with other sources of information. In any case a proper validation of results is needed to avoid the risk of large over-fitting of calibration equations.

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Conflict of interest

Authors have no conflicts of interest.

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Table 1

Descriptive statistics of productive traits, and milk and cheese composition of sampled cows according to different farming systems.

	3 FARMING SYSTEMS[a]:			5 FARMING SYSTEMS[a]:				
	Traditional[b]	Modern		Traditional		Modern		
							TMR	
		No-silage[c]	Silage[d]	No-AF	AF	No-TMR	No-silage[c]	Silage[d]
Farms, n	28	48	9	15	13	31	17	9
Cow/milk and cheese samples, n	420	714	130	225	195	461	253	130
Animal	tied	loose	loose	tied	tied	loose	loose	loose
Milking	at stalls	parlor	parlor	at stalls	at stalls	parlor	parlor	parlor
Major forage	hay	hay	hay/silage	hay	hay	hay	hay	hay/silage
Major concentrate	compound	compound	cereal mix	compound	compound	compound	cereal mix	cereal mix
Forage:concentrate	0.69:0.31	0.61:0.39	0.47:0.53	0.73:0.27	0.65:0.35	0.64:0.36	0.52:0.48	0.47:0.53
Productive traits:								
Milk yield, $kg \times d^{-1}$	20.6±6.9	26.0±7.6	27.4±8.2	19.4±7.1	22.1±6.3	24.7±7.0	28.3±8.1	27.4±8.2
Days in milk, d	179±114	180±109	176±109	175±119	184±108	184±110	174±106	176±109
Parity, n	2.86±1.99	2.62±1.64	2.55±1.54	2.82±1.97	2.90±2.03	2.74±1.73	2.42±1.43	2.55±1.54
Milk composition:								
Protein, g/100g	3.66±0.45	3.79±0.41	3.84±0.42	3.67±0.47	3.66±0.42	3.78±0.41	3.82±0.42	3.84±0.42
Fat, g/100g	4.21±0.78	4.39±0.89	4.94±1.07	4.16±0.82	4.26±0.74	4.38±0.94	4.41±0.77	4.94±1.07
SCS[e], U	2.96±2.03	3.02±1.77	2.84±1.79	3.07±2.04	2.83±2.02	3.13±1.74	2.83±1.80	2.84±1.79
pH	6.64±0.09	6.64±0.08	6.64±0.09	6.63±0.10	6.65±0.08	6.63±0.08	6.64±0.07	6.64±0.09
Cheese composition:								
Protein, g/100g	26.5±4.2	27.5±4.0	25.7±4.2	26.3±4.1	26.6±4.3	27.5±4.1	27.4±3.9	25.7±4.2
Fat, g/100g	38.5±4.2	37.6±4.3	39.9±4.7	38.5±4.2	38.6±4.3	37.7±4.3	37.5±4.4	39.9±4.7
pH	5.19±0.18	5.15±0.18	5.22±0.14	5.20±0.19	5.17±0.17	5.13±0.20	5.18±0.14	5.22±0.14

[a]AF = automatic feeders at mangers to control individually concentrate distribution; TMR = total mixed ration; modern TMR no silage = water added in the mixer wagon to enhance mixing; productive traits as well as milk and cheese composition are expressed as mean and standard deviation (in parenthesis); [b]Traditional = cluster of herds composed by traditional farming systems with and without AF; [c]Modern no silage = cluster of herds composed by modern farming systems with hay plus compound feed and modern TMR without silage; [d]Modern silage = cluster of herds that used TMR and corn silage; [e]SCS = $\log_2(SCC/100,000) + 3$.

Table 2

Main characteristics of the sources of information used for the authentication of milk and cheese from different farming systems.

	Milk:		Cheese:		
	FTIR spectrum (FTIRs)	Fatty acid profile (FAs)	NIR spectrum (NIRs)	Volatile fingerprint (VOCs)	Sensory profile (SENS)
Samples analyzed:					
Number of farms	85	83	62	72	83
Number of cows/samples	1,222	1,175	915	1,075	1,224
Sample used	50 mL	10 mL	30 g	3 g	80 g
Sample preparation	None	Methylation	Grinding	Grinding	Slicing
Chemicals used	No	Yes	No	No	No
Replicates per sample	20	1	16	1	6
Instrument used:					
Type	Infrared spectrometer	Gas chromatograph	Infrared spectrometer	PTR-ToF mass spectrometer	Trained sensory test panel
Denomination	FT 6000	ThermoQuest	FoodScan	PTR-ToF-MS 8000	-
Producer	Foss Electric A/S	Thermo Electron Corp.	Foss Electric A/S	Ionicon Analytik GmbH	DAFNAE
Address	Hillerød	Waltham	Hillerød	Innsbruck	Padova
Country	Denmark	USA	Denmark	Austria	Italy
Output obtained:					
Type	Absorbance	Fatty acids	Transmittance	VOCs	Descriptors
Unit	Log (T^{-1})	Percentage	Log (A^{-1})	ppb_v	Scores
Data per sample	1,060	47	100	619	47

Table 3

Summary of overall correct classification of milk or cheese samples from 3 or 5 dairy systems applying 10-fold cross-validation linear discriminant analysis and odds ratio of each source of information respect to FTIRs.

Source of information	Total Samples N.	Training			Testing		
		Samples N.	% Correct classification	Odds ratio[a]	Samples N.	% Correct classification	Odds ratio[a]
3 farming systems:							
FTIRs	1,222	972	97.4	1.00[b]	250	73.5	1.00[b]
FAs	1,175	940	81.1	**0.11**	235	77.3	**1.23**
FTIRs+FAs	1,130	903	99.6	**7.17**	227	77.9	**1.27**
NIRs	903	720	75.9	**0.08**	183	67.1	**0.74**
VOCs	1,075	860	83.1	**0.15**	215	66.9	**0.74**
NIRs+VOCs	767	614	94.0	**0.41**	153	71.5	0.96
SENS	1,224	970	89.1	**0.22**	254	60.2	**0.54**
5 farming systems:							
FTIRs	1,222	972	98.6	1.00[b]	250	65.0	1.00[b]
FAs	1,175	940	70.0	**0.03**	235	65.1	1.02
FTIRs+FAs	1,130	903	99.8	**5.43**	227	70.3	**1.28**
NIRs	903	720	66.8	**0.28**	183	52.1	**0.59**
VOCs	1,075	860	75.1	**0.04**	215	48.2	**0.49**
NIRs+VOCs	767	614	94.3	**0.26**	153	57.3	**0.72**
SENS	1,224	970	89.7	**0.12**	254	42.7	**0.40**

[a]The odds ratio in bold are characterized by 95% credibility regions not including 1.00 and then considered superior (>1.00) or inferior (<1.00) respect to reference (FTIRs); [b] = reference method.

Table 4

Correct classification and odds ratio estimates of milk or cheese samples from 3 farming systems applying 10-fold cross-validation linear discriminant analysis using the information obtained using the methods summarized in Table 2.

Source of information	Training			Testing		
	Samples N.	% Correct classification	Odds ratio[a]	Samples N.	% Correct classification	Odds ratio[a]
Traditional system:						
FTIRs	322	95.8	1.00[b]	83	67.9	1.00[b]
FAs	295	68.8	**0.10**	72	66.1	0.92
FTIRs+FAs	286	99.2	**5.94**	68	73.4	**1.30**
NIRs	264	70.0	**0.10**	71	61.8	**0.76**
VOCs	252	71.2	**0.11**	63	57.3	**0.63**
NIRs+VOCs	200	91.0	**0.44**	50	67.3	0.98
SENS	329	77.4	**0.15**	87	41.2	**0.33**
Modern without silage:						
FTIRs	545	98.0	1.00[b]	144	77.5	1.00[b]
FAs	546	86.7	**0.13**	139	82.4	**1.36**
FTIRs+FAs	525	99.8	**10.7**	135	79.8	1.14
NIRs	361	83.7	**0.11**	87	78.5	1.06
VOCs	522	90.4	**0.19**	133	77.1	0.98
NIRs+VOCs	329	95.4	**0.42**	83	74.8	0.98
SENS	550	94.6	**0.36**	142	81.0	**1.24**
Modern with silage:						
FTIRs	104	99.5	1.00[b]	24	69.7	1.00[b]
FAs	99	86.7	**0.03**	24	82.2	**2.00**
FTIRs+FAs	92	100.0	**9.99**	24	79.8	1.75
NIRs	95	62.1	**0.01**	25	43.4	0.34
VOCs	86	73.2	**0.11**	19	30.5	0.19
NIRs+VOCs	85	95.7	**0.11**	20	68.5	0.95
SENS	92	98.5	**0.32**	24	6.5	**0.03**

[a]The odds ratio in bold are characterized by 95% credibility regions not including 1.00 and then considered superior (>1.00) or inferior (<1.00) respect to reference (FTIRs); [b] = reference method.

Table 5

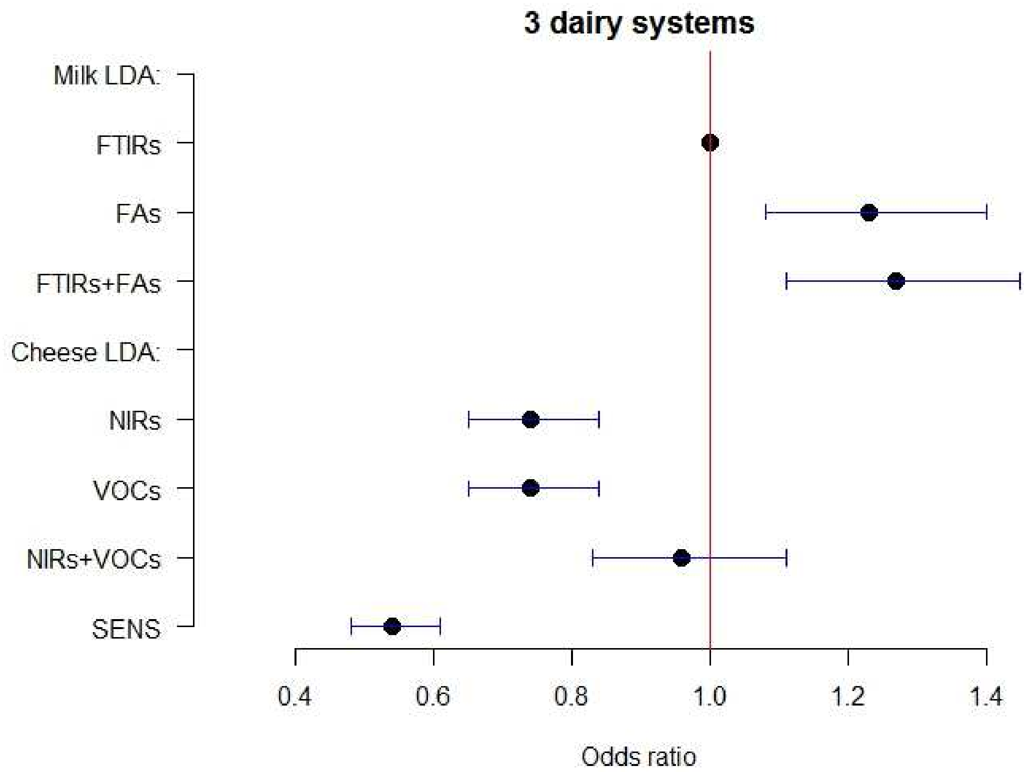
Correct classification and odds ratio estimates of milk or cheese samples from 5 farming systems applying 10-fold cross-validation linear discriminant analysis using the information obtained using the methods summarized in Table 2.

Source of information	Training			Testing		
	Samples N.	% Correct classification	Odds ratio[a]	Samples N.	% Correct classification	Odds ratio[a]
Traditional without AF[c]:						
FTIRs	172	99.7	1.00[b]	45	66.9	1.00[b]
FAs	163	62.2	**0.01**	39	60.4	0.76
FTIRs+FAs	158	100.0	4.61	36	75.7	**1.55**
NIRs	162	67.2	**0.01**	43	54.9	**0.60**
VOCs	104	69.5	**0.01**	31	39.5	**0.33**
NIRs+VOCs	110	92.4	**0.04**	25	55.5	**0.62**
SENS	175	90.4	**0.03**	46	39.7	**0.33**
Traditional with AF[c]:						
FTIRs	150	98.9	1.00[b]	38	54.6	1.00[b]
FAs	133	37.9	**0.01**	32	25.4	**0.28**
FTIRs+FAs	128	99.9	**13.79**	32	61.5	1.33
NIRs	102	46.4	**0.01**	28	28.5	**0.33**
VOCs	148	70.5	**0.03**	32	48.5	**0.28**
NIRs+VOCs	90	95.1	**9.69**	25	46.0	**0.71**
SENS	154	85.0	**0.06**	41	18.6	**0.67**
Modern: hay + compound feed:						
FTIRs	348	97.6	1.00[b]	95	66.3	1.00[b]
FAs	351	79.3	**0.09**	90	74.4	**1.48**
FTIRs+FAs	339	99.6	5.89	85	69.8	1.17
NIRs	239	77.5	**0.08**	58	70.8	1.24
VOCs	351	82.0	**0.11**	95	58.0	**0.70**
NIRs+VOCs	238	95.0	**0.46**	59	64.8	**0.40**
SENS	355	90.4	**0.23**	90	67.7	1.06
Modern: TMR[d] without silage:						
FTIRs	198	98.8	1.00[b]	48	67.1	1.00[b]
FAs	193	72.0	**0.03**	51	68.4	1.06
FTIRs+FAs	186	99.7	**3.79**	50	68.4	1.06
NIRs	122	61.7	**0.02**	29	42.7	**0.37**
VOCs	171	66.8	**0.03**	38	38.4	**0.31**
NIRs+VOCs	91	91.5	**0.13**	24	43.8	**0.38**
SENS	196	87.4	**0.09**	51	35.0	**0.26**
Modern: TMR[d] with silage:						
FTIRs	104	99.5	1.00[b]	24	69.3	1.00[b]
FAs	100	88.9	**0.04**	23	86.5	**2.86**
FTIRs+FAs	92	100.0	4.44	24	80.4	**1.83**
NIRs	95	67.6	**0.01**	25	43.6	**0.35**
VOCs	86	78.0	**0.02**	19	36.8	**0.26**
NIRs+VOCs	85	96.5	**0.13**	20	68.0	0.95
SENS	92	98.5	**0.31**	24	8.1	**0.04**

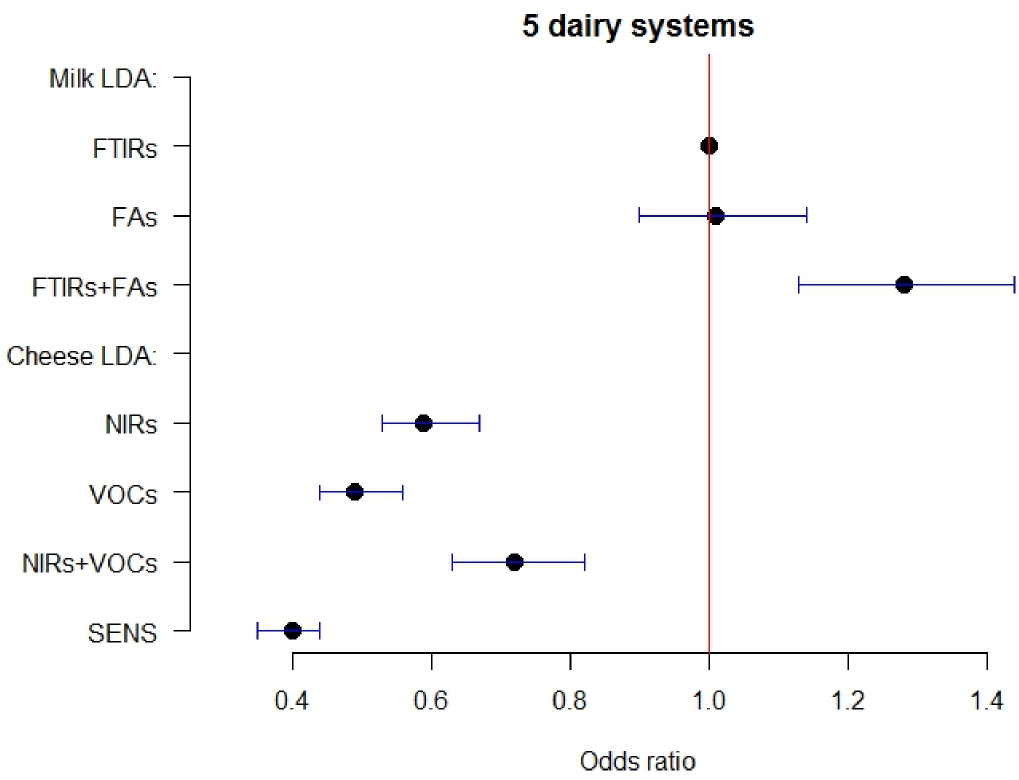
[a]The odds ratio in bold are characterized by 95% credibility regions not including 1.00 and then considered superior (>1.00) or inferior (<1.00) respect to reference (FTIRs); [b] = reference method. [c]AF = automatic feeder; [d]TMR = total mixer ration.

Figure 1.

[a]


3 dairy systems
Milk LDA:
FTIRs
FAs
FTIRs+FAs
Cheese LDA:
NIRs
VOCs
NIRs+VOCs
SENS
0.4
0.6
0.8
1.0
1.2
1.4
Odds ratio

[b]


5 dairy systems
Milk LDA:
FTIRs
FAs
FTIRs+FAs
Cheese LDA:
NIRs
VOCs
NIRs+VOCs
SENS
0.4
0.6
0.8
1.0
1.2
1.4
Odds ratio

Caption

Figure 1.

Odds ratio estimates and credibility region (95%) for the correct classification of milk or cheese samples from three [**a**] and five [**b**] dairy systems applying 10-fold cross-validated linear discriminant analysis respect to FTIRs considered as reference method (odds ratio = 1.00).

Supplementary materials

Supplementary Figure S1.

Figure S1. Principal component analysis (PCA) of FTIR spectra measured from 1223 milk sample collected in dairy farms classified as traditional farms, modern farms without the use of silages, and modern farms using silages. Components 1 and 2 explain 34.30 and 8.56% of the variance, respectively.

Supplementary Figure S2.

Figure S1. Principal component analysis (PCA) of NIRS spectra measured from 904 cheese sample originated from milk sample collected in dairy farms classified as traditional farms, modern farms without the use of silages, and modern farms using silages. Components 1 and 2 explain 70.29 and 24.97% of the variance, respectively.