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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

81 *2.1. Experimental setup*

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

98 *2.2. Milk sampling*

99 On a given day, only 1 herd (generally 15 cows per herd) was sampled during the evening
100 milking. Two milk subsamples were taken from each cow and immediately refrigerated at 4 °C
101 without any preservative. One subsample (50 mL) was taken to the milk quality laboratory of the
102 Breeders Federation of the Province of Trento (Trento, Italy) for chemical composition analysis.
103 The other subsample (2,000 mL) was taken to the cheese-making laboratory of the Department of
104 Agronomy, Food, Natural Resources, Animals and Environment of the University of Padova
105 (Legnaro, Italy); an aliquot (1,500 mL) of this subsample was used for cheese production, and the

106 remainder was analyzed for its fatty acid profile. All samples were processed for analysis and model
107 cheese manufacture within 20 h of collection (Cecchinato and Bittante, 2016).

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

114 *2.3. FTIR analysis*

115 All individual milk samples were analyzed by Fourier-transform infrared spectroscopy
116 (MilkoScan FT6000, Foss, Hillerød, Denmark) over the spectral range from wavenumber 5,011 to
117 925 cm^{-1}, corresponding to wavelengths 2,000 to 10,800 nm, yielding a total of 1,060 waves (Table
118 2), according to the procedure described by Ferragina et al. (2015). Spectra were stored as
119 absorbances (A) using the transformation A = log(1/T), where T is the transmittance. Before data
120 analysis, 10 replicate spectra obtained from 2 aliquots averaged to obtain one spectrum for each
121 milk sample.

122 *2.4. Gas chromatography analysis*

123 Fatty acid analysis was performed as described in detail by Mele et al. (2016). Briefly, each
124 milk sample was centrifuged at 5,000 × g for 30 min at 4 °C. Thirty milligrams of fat were collected
125 in a vial, and mixed with 3 mL of hexane and 0.3 mL of 2 M methanolic solution of KOH. The
126 mixture was incubated for 5 min at room temperature after the addition of 0.25 mg of $NaHSO_3$ ×
127 H_2O. The samples were then centrifuged at 3,000 × g for 3 min at 4 °C, and the upper layer was
128 collected for GC analysis. Fatty acid composition was determined using a ThermoQuest gas
129 chromatograph (ThermoElectron Corp., Waltham, MA, USA) equipped with a flame-ionization
130 detector and a high polar fused-silica capillary column (Chrompack CP-Sil88 Varian, Middelburg,
131 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

158 waves. From every cheese sample, 16 replicated entire spectra were acquired and averaged before
159 statistical analysis.

160 *2.7. PTR-ToF-MS analysis*

161 Volatile organic compound analysis was performed as described by Bergamaschi et al.
162 (2015 and 2016). Briefly, 3 g of each cheese sample, hitherto stored at -80 °C, were thawed at 20 °C
163 for 6 h then placed in glass vials (20 ml, Supelco, Bellefonte, USA), capped with PTFE/Silicone
164 septa (Supelco), then measured with a PTR-ToF-MS 8000 instrument (Ionicon Analytik GmbH,
165 Innsbruck, Austria) (Table 2). The conditions in the drift tube of the PTR were as follows:
166 temperature 110 °C, drift pressure 211 Pa, drift voltage 500 V. Internal calibration and
167 spectrometric peak extraction were performed according to the procedures described by Cappellin
168 et al. (2010, 2012), resulting in the identification of 619 spectrometric peaks per cheese sample.
169 Headspace volatile organic compound concentrations, expressed as parts per billion by volume,
170 were estimated using the method described by Lindinger, Hansel, & Jordan (1998).

171 *2.8. Sensory profile*

172 Sensory analysis was performed by a trained panel as described in detail by Cipolat-Gotet et
173 al. (2018), while the reference standard and the protocol scorecard were in accordance with
174 Bérodier et al. (1997) and Lavanchy et al. (1993). Briefly, 14 panelists (6 females and 8 males, age
175 35.6±11.8 years) were selected and trained in cheese evaluation under the direction of a panel
176 leader. Their task was to assess the cheese samples according to 7 main sensory descriptors:
177 intensity of smell, intensity of flavor, intensity of salt and sour tastes, elasticity, firmness, and
178 moisture. These traits were ranked on a 13-point discontinuous scale (from 1 to 7, including half
179 points). A further level of sensory description was introduced for smell and flavor: after assessing
180 their overall intensities, the assessors had to evaluate on a 4-point discontinuous scale the intensities
181 of 4 families of descriptors, each composed of several detailed attributes, giving a total of 20 traits
182 for smell and 20 for flavor.

183 *2.9. Statistical analysis*

184 *2.9.1. Data processing*

185 The absorbance values of every wavelength in the FTIR spectra (1,060 waves) of the milk
186 (1,222 samples), and in the NIR spectra (100 waves) of the cheese (915 samples) were centered and
187 standardized to a null mean and a unit sample variance. Next, we calculated Mahalanobis distances
188 using the standardized FTIR and NIR spectra data to detect outliers. Having decided from
189 examining the plot to exclude only spectra with a very high probability of being outliers, we
190 discarded those with a Mahalanobis distance greater than 3 times the standard deviation. To
191 increase the normality of distribution of absorbance value and improve LDA, first-derivative
192 Savitzky-Golay was also applied to the spectra as a mathematical pretreatment (Savitzky & Golay,
193 1964) for both FTIR spectra of milk and NIR spectra of cheese.

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

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

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

208 *2.9.2. Linear discriminant analysis*

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

225 *2.9.3. Comparison of discriminant ability of LDA models by logistic regression*

226 The discriminating ability of a model is usually evaluated through a graphical representation
227 of data of each model. In this case, the large number of models tested (5 sources of information + 2
228 combination × 2 number of farming systems to be discriminated = 14 LD analyses), the large
229 number of samples analyzed (915 to 1,124 per source) and the possibility to plot only two latent
230 variables make unfeasible this approach. To test the differences in the discrimination ability of the
231 14 models compared here considering the specific distribution and variability of each source of
232 information, a logistic regression was carried out. Correct classification rates for all the LDA
233 models were coded as binary variables (0, 1), where 1 indicated correct classification of the milk or

234 cheese sample. This new variable was analyzed by logistic regression using the SAS LOGISTIC
235 procedure (SAS Institute, 2012) according to the following model:

236 $$\text{logit}(\pi) = \log\left(\frac{\pi}{1-\pi}\right) - \alpha + \beta' x_1$$

237 where $\pi = \Pr(Y = 1|x)$, which is the response probability (odds ratio) of correct
238 classification; α is the intercept of the parameter; $\beta = (\beta_1, \ldots, \beta_i)$, which is the vector of the i slope
239 parameters; and x is a vector for the fixed effects of the methods: source of information (7 levels),
240 dairy system (3 or 5 levels), and the source-dairy system interaction (21 or 35 levels). The odds
241 ratio estimates together with their confidence intervals for each source of information were used to
242 plot these across the different dairy systems (Figure 1 a and b). For a better understanding of the
243 nature of the information available through the infrared spectra, a principal component analysis was
244 carried out on the milk FTIR spectra and on cheese NIR spectra.
245
246 **3. Results and discussion**
247 Only few published papers compared different instruments with the same pool of samples,
248 and none compared, as the present study does, several sources of information on a large scale to
249 discriminate milk and cheese according to the farming system in which cows were reared.
250 Scampicchio et al. (2016) studied the possibility of discriminating 189 milk samples using 4 sources
251 of information and their combination: chemical components of milk predicted from FTIR spectra
252 (they did not used the absorbance values for discrimination); NIR absorbance spectra; FA profiles
253 from GC; and stable isotopes ($^{13}C/^{12}C$ and $^{15}N/^{14}N$). They aimed at discriminating milk samples
254 according to region (40 samples from North Tyrol, 130 from South Tyrol and 19 from other origin
255 "Europa"), season (71 spring, 42 summer, 14 autumn, 43 winter, and 19 unknown) and heat
256 treatment (90 raw milk, 77 HTST, and 19 UHT). Using a principal component analysis, they
257 obtained very poor results whether using each one of the four sources of information for
258 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

310 during testing, showing that a large number of data points is not strictly necessary and it can cause
311 over-fitting during calibration.
312 It is worthwhile of noting that McQueen et al. (1995) compared NIR and MIR spectroscopic
313 techniques (different from ours) to predict the protein, fat and moisture contents of cheese, and
314 found prediction by NIR spectroscopy to be more precise.
315 Obviously, the differences may also be explained by the fact that we sampled the milk on the
316 farm at the time of milking, and analyzed it within a few hours, during which time the sample was
317 kept sealed and refrigerated. This means that it fully reflects the conditions of the different farming
318 systems considered and the individual farms within system. The model cheeses were all made in the
319 same laboratory following a standardized procedure (Cipolat-Gotet et al., 2013) with the aim of
320 controlling all external sources of variation as much as possible. The cheeses were then sampled
321 after 2 months of ripening, during which many physical, biochemical and microbiological changes
322 had taken place (Fox et al., 2004). We then analyzed them to look after two months for residual
323 effects of the differences in the raw materials from which they were manufactured. Interestingly for
324 dairy industry, our results show that after 2 months of ripening the cheeses still exhibited the effects
325 of farming system on the raw materials (milk) from which they were made (Table 3).
326 As supportive evidence based on multivariate analysis are available for milk fatty acid
327 profile (Mele et. al., 2016) and volatile organic profile of cheese (Bergamaschi et al., 2015), but not
328 for infrared spectra, the main results of a principal component analysis was carried out also on the
329 milk FTIR spectra and on cheese NIR spectra and the results are available as supplementary
330 material (Supplementary Figures S1 and S2). Principal component analysis is a useful instrument
331 for understanding the relationships among many variables, like infrared absorbencies, to reduce the
332 dimensionality of a large database, and also to visualize different groups of samples (O'Callaghan
333 et al., 2017), but it is also criticized because it provides only a qualitative view of the data and it is
334 not specific for discriminating analyses (Granato et al., 2018b).
335

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

385 The overall results of the 10-fold cross-validated LDAs using the NIR spectra and VOCs of
386 cheese are given in Table 3. When discriminating between the 3 farming systems, we found the
387 average correct classification rate in training to be greater with VOCs (83.1%) than NIRS (75.9%).
388 The results of the cross-validation show that both techniques had lower rates of correct
389 classification and were similar to each other (NIRS, 67.1%; VOCs, 66.9%). Combining the two
390 sources of information (NIRS+VOCs) increased the levels of correct classification of the cheese
391 samples during the training analysis (94.3%), and also during cross-validation, although to a lesser
392 degree (71.5%) (Table 3). When we tried to discriminate the cheese samples from the 5 different
393 farming systems, the results of the LDA of the training subset revealed lower percentages for NIRS
394 (66.8%) and for VOCs (75.1%), but not for NIRS+VOCs (95.3%), compared with discrimination of
395 cheese samples from the 3 farming systems. With regards to the testing subset, we observed a
396 decrease in the overall rates of correct classification of cheese samples with both methods
397 individually (52.1% for NIRS, 48.2% for VOCs), and also in combination (57.3%).
398 The results of the LDAs for discriminating each individual farming system from cheese
399 characteristics are reported in Tables 4 and 5. In particular, the rate of correct classification of
400 cheese samples based on VOCs and the training dataset was higher than that based on NIRS, for
401 both the 3- and 5-dairy system classifications. The LDAs based on NIRS and VOCs yielded slightly
402 better results with the training datasets than with the testing datasets, for all 3 or 5 farming systems.
403 It is well documented that infrared spectra reflect the chemical compositions of specific milk
404 and cheese samples, and they have been utilized mainly for prediction purposes (Wojciechowski
405 and Barbano, 2016; Margolies and Barbano, 2017). NIRS has also been used for discrimination
406 purposes, mainly to distinguish between cheese samples derived from pasture-based *vs.* silage-
407 based systems, and derived from hay *vs.* silage-based systems. Andueza, Agabriel, Constant, Lucas,
408 & Martin, (2013) reported classification rates higher than 90% when discriminating Abondance (n
409 = 92), Tomme de Savoie (n = 107) and Cantal cheeses (n = 109) obtained from the milk of cows fed
410 at pasture or on preserved forage using a 4-fold cross-validated partial least square discriminant

411 analysis. The slightly lower rate of correct classification of the samples in our study is clearly a
412 consequence of not having included pasture-based systems. Support for this explanation comes
413 from reports (Martin, et al., 2005; Valenti et al., 2013) of the difficulty of distinguishing between
414 dairy products derived from cows fed on hay and from cows fed on maize silage using spectra and
415 terpenes as variables. It could be interesting, at industry level, the fact that flavor profile could be
416 used to discriminate cheeses in relation to the residual effect of the farming system of the milk used
417 for cheese-making, even after two months of ripening. But it is also of interest the fact that VOCs
418 profile is not superior to NIRS spectrum in this regard.

419

420 *3.4. Sensory traits for discriminating cheese samples*

421 The results of the 10-fold cross-validated LDA using the sensory traits (SENS) of cheese are
422 reported in Table 3. Whether discriminating between the 3 or the 5 farming systems, we found the
423 average rate of correct classification in training with SENS (89.1% and 89.7%, respectively) to be
424 much greater than with NIRs and VOCs, but much lower with the combination NIRS+VOCs
425 (94.0% for the 3-system group, 94.3% for the 5-system group). On the contrary, the results of the
426 cross-validation showed that a smaller percentage of samples were correctly classified by SENS
427 than by any of the instrumental sources of information, as clearly shown in Figure 1. With respect
428 to the individual farming systems, the rates of correct classification of cheese samples by SENS
429 with the training datasets were very similar for all the farming systems (Tables 4 and 5) and
430 particularly low when discriminating cheeses derived from cows fed on silages during cross-
431 validation (Table 4 and 5).

432 Using the same model cheeses as in this study, we recently found that those derived from
433 farms using silage were perceived as having greater firmness and less moisture (Cipolat-Gotet et al.,
434 2018). Other authors (e.g., Martin et al., 2005) also reported that cheese was influenced by the
435 presence or absence of maize silage in the cows' diets. However, we also found a large effect of

436 individual herd within dairy system on 47 sensory descriptors, which varied from just under 15% to
437 70%. The other large source of variation observed in our previous study was the panelists.
438 Evidently, the large variation among different types of farm and among panelists precludes
439 obtaining a high rate of discrimination according to dairy system using the sensory profiles of
440 cheeses. So, this methodology could be interesting as a research tool, but not at level of the dairy
441 industry.

442 *3.5. Future application of food authentication*

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

454 The methods used on fluid milk (FTIR spectrum and GC FA profile) could be used to
455 control the bulk milk supplied to dairies by individual farmers or commercial traders or the
456 packaged milk supplied to retailers' chains by different dairies. The use of FTIR spectra for dairy
457 system authentication is much more practical and less expensive than GC FAs, and could be used
458 for characterizing milk for many chemical composition traits (ISO-IDF (2013) and technological
459 properties (Bittante et al., 2012). On the other hand, FAs could be used also for a better certification
460 of nutritional properties of milk in relation to human health (Shingfield et al., 2013; Mele et al.,
461 2016).

462 The instrumental methods used for cheese authentication (NIR spectrum and VOCs profile)
463 have demonstrated that also after 2-month ripening, still some influence of dairy farming system of
464 origin of milk could be captured. In this case the possible interest is at level of cheese factory for
465 monitoring cheese batches obtained from milk of different suppliers, but also at level of different
466 PDO consortia or retailer chains for comparing different cheese producers. If, also in this case, the
467 use of NIR spectra is much more practical and less expensive (Andueza et al., 2018), the use of
468 VOCs profile could offer other information and certification of cheese flavor and possibly replace
469 the use of sensory description actually mandatory according to the norms of production of some
470 PDO cheeses (Bittante et al., 2011a and b; Ojeda et al., 2015).
471
472 **4. Conclusions**
473 In this study, we compared different sources of information for discriminating different
474 farming systems from a large number of individual milk samples and from the individual model
475 cheeses obtained from processing these same milk samples according to a standardized procedure.
476 Our findings with regards to discriminating different farming systems were that: a) the results from
477 the training subsets (calibration) were often high, especially for those sources of information with
478 many data points; b) the results from the testing subsets (validation) were lower, and not much
479 influenced by the number of data points available; c) discrimination between a larger number (5) of
480 farming systems tended to be less efficient than between fewer (3) farming systems; d) the
481 information from instrumental techniques is more effective than that obtained from the sensory
482 descriptors developed by trained panelists; e) the information from instrumental techniques for
483 analyzing milk was more effective than that from techniques for analyzing cheese, although after
484 two months of ripening the cheeses still showed the influence of farming system; f) fatty acid
485 profiles tended to be more effective than infrared spectra for milk sample validation, and combining
486 them further increases discrimination ability; g) infrared spectra and volatile fingerprints are equally
487 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.

References

Abbas, O., Zadravec, M., Baeten, V., Mikuš, T., Lešić, T., Vulić, A., Prpić, J., Jemeršić, L., Pleadin, J. (2018). Analytical methods used for the authentication of food of animal origin. *Food Chemistry*, *246*, 6-17.

Andueza, D., Agabriel, C., Constant, I., Lucas, A., & Martin, B. (2013). Using visible or near infrared spectroscopy (NIRS) on cheese to authenticate cow feeding regimes. *Food Chemistry,*

512 *141,* 209-214.

513 Bergamaschi, M., Biasioli, F., Cappellin, L., Cecchinato, A., Cipolat-Gotet, C., Cornu, A., …
514 Bittante, G. (2015). Proton transfer reaction time-of-flight mass spectrometry: A high-
515 throughput and innovative method to study the influence of dairy system and cow
516 characteristics on the volatile compound fingerprint of cheeses. *Journal of Dairy Science,*
517 *98,* 8414-8427.

518 Bergamaschi, M., Cecchinato, A., Biasioli, F., Gasperi, F., Martin, B., & Bittante, G. (2016a). From
519 cow to cheese: Genetic parameters of the flavour fingerprint of cheese investigated by direct-
520 injection mass spectrometry (PTR-ToF-MS). *Genetics Selection Evolution,* 48:89.

521 Bergamaschi, M., Cipolat-Gotet, C., Stocco, G., Valorz, C., Bazzoli, I., Sturaro, E., Ramanzin, M.,
522 & Bittante, G. (2016b). Cheese making in highland pastures: Milk technological properties,
523 cream, cheese and ricotta yields, milk nutrient recovery, and products composition. *Journal of*
524 *Dairy Science. 99,* 9631–9646.

525 Bérodier, F., Lavanchy, P., Zannoni, M., Casals, J., Herrero, L., & Adamo, C. (1997). Guide
526 d'Évaluation Olfacto-Gustative des Fromages à Pâte Dure et Semi-dure. LWT - *Food Science*
527 *and Technology, 30,* 653-664.

528 Bertoni, G., Calamari, L., Maianti, M. G., & Battistotti B. (2005). Milk for Protected Denomination
529 of Origin (PDO) cheeses: I. The main required features. Pages 217–228 in *Indicators of Milk*
530 *and Beef Quality*. J. F. Hocquette and S. Gigli, ed. EAAP publication 112. Wageningen
531 Academic Publishers, Wageningen, the Netherlands.

532 Bittante, G., & Cecchinato, A. (2013). Genetic analysis of the Fourier transform infrared spectra of
533 bovine milk with emphasis on individual wavelengths related to specific chemical bonds.
534 *Journal of Dairy Science, 96,* 5991-6006.

535 Bittante, G., Cipolat-Gotet C., Malchiodi F., Sturaro E., Tagliapietra F., Schiavon S., & Cecchinato
536 A. (2015). Effect of dairy farming system, herd, season, parity, and days in milk on

537 modeling of the coagulation, curd firming, and syneresis of bovine milk. *Journal of Dairy*
538 *Science, 98,* 2759-2774.
539 Bittante, G., Cecchinato, A., Cologna, N., Penasa, M., Tiezzi, F., & De Marchi, M.. (2011a).
540 Factors affecting the incidence of first-quality wheels of Trentingrana cheese. *Journal of Dairy*
541 *Science, 94,* 3700–3707.
542 Bittante, G., Cipolat-Gotet C., Malchiodi F., Sturaro E., Tagliapietra F., Schiavon S., & Cecchinato
543 A. (2015). Effect of dairy farming system, herd, season, parity, and days in milk on
544 modeling of the coagulation, curd firming, and syneresis of bovine milk. *Journal of Dairy*
545 *Science, 98,* 2759-2774.
546 Bittante, G., Cologna, N., Cecchinato, A., De Marchi, M., Penasa, M., Tiezzi, F., Endrizzi, I., &
547 Gasperi, F. (2011b). Monitoring of sensory attributes used in the quality payment system of
548 Trentingrana cheese. *Journal of Dairy Science, 94,* 5699–5709.
549 Bittante, G., Penasa, M., & Cecchinato, A. (2012). Invited review: Genetics and modeling of milk
550 coagulation properties. *Journal of Dairy Science, 95,* 6843-6870.
551 Bovolenta, S., Romanzin, A., Corazzin, M., Spanghero, M., Aprea, E., Gasperi, F., & Piasentier, E.
552 (2014). Volatile compounds and sensory properties of Montasio cheese made from the milk of
553 Simmental cows grazing on alpine pastures. *Journal of Dairy Science, 97,* 7373-85.
554 Buchin, S., Martin, B., Dupont, D., Bornard, A., & Achilleos, C. (1999). Influence of the
555 composition of Alpine highland pasture on the chemical, rheological and sensory properties
556 of cheese. *Journal of Dairy Research, 66,* 579-588.
557 Cecchinato, A., & Bittante G. (2016). Genetic and environmental relationships of different
558 measures of individual cheese yield and curd nutrients recovery with coagulation properties
559 of bovine milk. *Journal of Dairy Science, 99,* 1975-1989.
560 Caligiani, A., Marseglia, A., & Palla, G. (2014). An overview on the presence of cyclopropane fatty
561 acids in milk and dairy products. *Journal of Agricultural and Food Chemistry, 62,* 7828-7832.
562 Cappellin, L., Biasioli, F., Fabris, A., Schuhfried, E., Soukoulis, C., Märk, T. D., & Gasperi, F.

563 (2010). Improved mass accuracy in PTR-TOF-MS: Another step towards better compound
564 identification in PTR-MS. *International Journal of Mass Spectrometry, 290,* 60-63.
565 Cappellin, L., Karl, T., Probst, M., Ismailova, O., Winkler, P. M., Soukoulis, C.,… Biasioli, F.
566 (2012). On Quantitative Determination of Volatile Organic Compound Concentrations Using
567 Proton Transfer Reaction Time-of-Flight Mass Spectrometry. *Environmental Science &*
568 *Technology, 46,* 2283-2290.
569 Capuano, E., Van Der Veer, G., Boerrigter-Eenling, R., Elgersma, A., Rademaker, J., Sterian, A., &
570 Van Ruth, S. M. (2014). Verification of fresh grass feeding, pasture grazing and organic
571 farming by cows farm milk fatty acid profile. *Food Chemistry, 164,* 234-241.
572 Cipolat-Gotet, C., Cecchinato, A., Drake, M. A., Marangon, A., Martin, B., & Bittante, G. (2018).
573 From cow to cheese: novel phenotypes related to the sensory profile of model cheeses from
574 individual cows. *Journal of Dairy Science, 101,* 5865-5877.
575 Coppa, M., Chassaing, C., Ferlay, A., Agabriel, C., Laurent, C., Borreani, G.,… Martin, B. (2015).
576 Potential of milk fatty acid composition to predict diet composition and authenticate feeding
577 systems and altitude origin of European bulk milk. *Journal of Dairy Science, 98,* 1539-1551.
578 Coppa, M., Martin, B., Agabriel, C., Chassaing, C., Sibra, C., Constant, I.,... Andueza, D. (2012).
579 Authentication of cow feeding and geographic origin on milk using visible and near-infrared
580 spectroscopy. *Journal of Dairy Science, 95,* 5544-5551.
581 Coppa, M., Ferlay, A., Monsallier, F., Verdier-Metz, I., Pradel, P., Didienne, R., Farruggia, A.,
582 Montel, M.C., & Martin, B. (2011). Milk fatty acid composition and cheese texture and
583 appearance from cows fed hay or different grazing systems on upland pastures. *Journal of*
584 *Dairy Science, 94,* 1132–1145.
585 Cossignani, L., Pollini, L., and Blasi, F. (2019). Invited review: Authentication of milk by direct
586 and indirect analysis of triacylglycerol molecular species. *Journal of Dairy Science 102. In*
587 *press*. https://doi.org/10.3168/jds.2019-16318.
588 Esteki, M., Simal-Gandara, J., Shahsavari, Z., Zandbaaf, S., Dashtaki, E., Vander Heyden, Y.

589 (2018). A review on the application of chromatographic methods, coupled to chemometrics,
590 for food authentication. *Food Control*, *93*, 165-182.
591 Ferlay, A., Agabriel, C., Sibra, C., Journal, C., Martin, B., & Chilliard, Y. (2008). Tanker milk
592 variability in fatty acids according to farm feeding and husbandry practices in a French semi-
593 mountain area. *Dairy Science and Technology, 88,* 193-215.
594 Ferragina, A., de los Campos, G., Vazquez, A., Cecchinato, A., & Bittante, G. 2015. Bayesian
595 regression models outperform partial least squares methods for predicting milk components
596 and technological properties using infrared spectra data. *Journal of Dairy Science, 98,* 8133-
597 8152.
598 Fox, P. F., McSweeney, P. L. H., Cogan, T. M., & Guinee, T. P. 2004. Cheese: Chemistry, Physics
599 and Microbiology. Vol. 1. General Aspects. 3rd ed. Elsevier/Academic Press, London, United
600 Kingdom.
601 González-Martín, I., Hernández-Hierro, J. M., Salvador-Esteban, J., González-Pérez, C., Revilla, I.,
602 & Vivar-Quintana, A. (2011). Discrimination of seasonality in cheeses by near-infrared
603 technology. *Journal of the Science of Food and Agriculture, 91,* 1064-1069.
604 Granato, D., Putnik, P., Bursać Kovačević, D., Sousa Santos, J., Calado, V., Silva Rocha, R.,
605 Gomes Da Cruz, A., Jarvis, B., Ye Rodionova, O., and Pomerantsev, A. (2018a). Trends in
606 chemometrics: food authentication, microbiology, and effects of processing. *Comprehensive*
607 *Reviews in Food Science and Food Safety, 17, 663-677.*
608 Granato, D., Santos, J.S., Escher, G.B., Ferreira, B.L., & Maggio, R.M. (2018b). Use of principal
609 component analysis (PCA) and hierarchical cluster analysis (HCA) for multivariate association
610 between bioactive compounds and functional properties in foods: A critical perspective. *Food*
611 *Science & Technology, 72,* 83-90.
612 Hurtaud, C., Dutreuil, M., Coppa, M., Agabriel, C., & Martin, B. (2014). Characterization of milk
613 from feeding systems based on herbage or corn silage with or without flaxseed and
614 authentication through fatty acid profile. *Dairy Science and Technology, 94,* 103-123.

615 ICAR, International committee for animal recording. 2012. International agreement of recording
616 practices. Guidelines approved by the general assembly held in Cork, Ireland on June 2012.
617 ICAR. Rome. Italy.
618 ISO-IDF. (2013). Milk and liquid milk products. Determination of fat, protein, lactose, pH, and
619 NaCl content. International Standard ISO 9622 and IDF 141:2013. *International Organization*
620 *for Standardization*, Geneva, Switzerland, and International Dairy Federation, Brussels,
621 Belgium.
622 Jiménez-Carvelo, A.M., González-Casado, A., Bagur-González, M.G., Cuadros-Rodríguez, L.
623 (2019). Alternative data mining/machine learning methods for the analytical evaluation of
624 food quality and authenticity–A review. *Food Research International*, *122*, 25-39.
625 Lavanchy, P., Bérodier, F., Zannoni, M., Noel, Y., Adamo, C., Squella, J., & Herrero, L. (1993).
626 Sensory evaluation of the texture of hard and semi-hard cheeses. *Lebensm. Wiss. Technol.*
627 *26,* 59-68.
628 Lindinger, W., Hansel, A., & Jordan, A. (1998). On-line monitoring of volatile organic compounds
629 at pptv levels by means of proton-transfer-reaction mass spectrometry (PTR-MS) medical
630 applications, food control and environmental research. *International Journal of Mass*
631 *Spectrometry and Ion Processes, 173,* 191-241.
632 Liu, N., Parra H. A., Pustjens, A., Hettinga, K., Mongondry, P., & van Ruth, S. M. (2018).
633 Evaluation of portable near-infrared spectroscopy for organic milk authentication. *Talanta,*
634 *184,* 128-135.
635 Lucas, A., Andueza, D., Ferlay, A., & Martin, B. (2008). Prediction of fatty acid composition of
636 fresh and freeze-dried cheeses by visible-near-infrared reflectance spectroscopy. *International*
637 *Dairy Journal, 18,* 595-604.
638 Margolies, B. J., & Barbano, D. M. (2017). Determination of fat, protein, moisture, and salt content
639 of Cheddar cheese using mid-infrared transmittance spectroscopy. *Journal of Dairy Science,*
640 *101,* 924-933.

641 Martin, B., Verdier-Metz, I., Buchin, S., Hurtaud, C., & Coulon, J.-B. (2005). How do the nature of
642 forages and pasture diversity influence the sensory quality of dairy livestock products? *Animal*
643 *Science, 81,* 205-212.
644 McQueen, D. H., Wilson, R., Kinnunen, A., & Jensen, E. P. (1995). Comparison of two infrared
645 spectroscopic methods for cheese analysis. *Talanta, 42,* 2007-2015.
646 Medina, S., Perestrelo, R., Silva, P., Pereira, J.A.M., Câmara, J.S. (2019). Current trends and recent
647 advances on food authenticity technologies and chemometric approaches. *Trends in Food*
648 *Science & Technology*, *85*, 163-176.
649 Mele, M., Macciotta, N. P. P., Cecchinato, A., Conte, G., Schiavon, S., & Bittante, G. (2016).
650 Multivariate factor analysis of detailed milk fatty acid profile: Effects of dairy system, feeding,
651 herd, parity, and stage of lactation. *Journal of Dairy Science, 99,* 9820-9833.
652 O'Callaghan, T.O., Mannion, D.T., Hennessy,D., McAuliffe, S., O'Sullivan, M.G., Leeuwendaal,
653 N., Beresford, T.P., Dillon, P., Kilcawley, K.N., Sheehan, J.J., Ross, R.P., & Stanton, C.
654 (2017). Effect of pasture versus indoor feeding systems on quality characteristics, nutritional
655 composition, and sensory and volatile properties of full-fat Cheddar cheese. *Journal of Dairy*
656 *Science, 100,*6053-6073.
657 Ojeda, M., Etaio, I., Fernandez Gil, M.P., Albisu, M., Salmeron, J., Pérez Elortondo, F.J. (2015).
658 Sensory quality control of cheese: Going beyond the absence of defects. *Food Control, 51,*
659 371-380.
660 SAS Institute. 2012. SAS/STAT Software. Release 9.4. SAS Institute Inc., Cary, NC.
661 Savitzky, A., & Golay, M. J. E. (1964). Smoothing and Differentiation of Data by Simplified Least
662 Squares Procedures. *Analytical Chemistry, 36,* 1627-1639.
663 Scampicchio, M., Eisenstecken, D., De Benedictis, L., Capici, C., Ballabio, D., Mimmo, T.,…
664 Cesco, S. (2016). Multi-method Approach to Trace the Geographical Origin of Alpine Milk: a
665 Case Study of Tyrol Region. *Food Analytical Methods, 9,* 1262-1273.
666 Shingfield, K.J. Bonnet, M., & Scollan, N.D. (2013). Recent developments in altering the fatty acid

composition of ruminant-derived foods. *Animal, 7:s1,* 132–162.

Slots, T., Butler, G., Leifert, C., Kristensen, T., Skibsted, L. H., & Nielsen, J. H. (2009). Potentials to differentiate milk composition by different feeding strategies. *Journal of Dairy Science, 92,* 2057-2066.

Stocco, G., Cipolat-Gotet C., Bobbo T., Cecchinato A. & Bittante G. (2017). Breed of cow and herd productivity affect milk composition and modeling of coagulation, curd firming and syneresis. *Journal of Dairy Science, 100,* 129-145.

Tornambé, G., Cornu, A., Pradel, P., Kondjoyan, N., Carnat, A. P., Petit, M., & Martin, B. (2006). Changes in Terpene Content in Milk from Pasture-Fed Cows. *Journal of Dairy Science, 89,* 2309.2319.

Valdés, A., Beltrán, A., Mellinas, C., Jiménez, A., Garrigós, M.C. (2018). Analytical methods combined with multivariate analysis for authentication of animal and vegetable food products with high fat content. *Trends in Food Science & Technology*. *77,* 120-130.

Valenti, B., Martin, B., Andueza, D., Leroux, C., Labonne, C., Lahalle, F.,... Ferlay, A. (2013). Infrared spectroscopic methods for the discrimination of cows' milk according to the feeding system, cow breed and altitude of the dairy farm. *International Dairy Journal, 32,* 26-32.

Wojciechowski, K. L., & Barbano, D. M. (2016). Prediction of fatty acid chain length and unsaturation of milk fat by mid-infrared milk analysis. *Journal of Dairy Science, 99,* 8561-8570.

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×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.

693 **Table 2**

694 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

695

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.

710 **Table 4**
711 Correct classification and odds ratio estimates of milk or cheese samples from 3 farming systems
712 applying 10-fold cross-validation linear discriminant analysis using the information obtained using
713 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**

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

716 **Table 5**

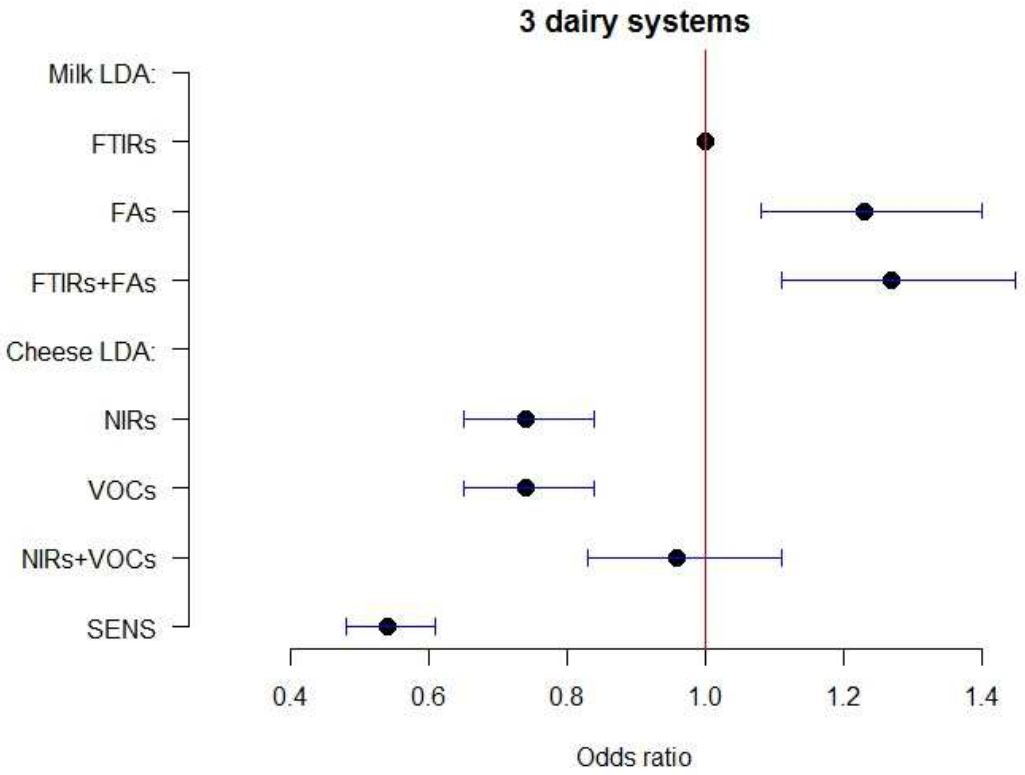
717 Correct classification and odds ratio estimates of milk or cheese samples from 5 farming systems
718 applying 10-fold cross-validation linear discriminant analysis using the information obtained using
719 the methods summarized in Table 2.

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**

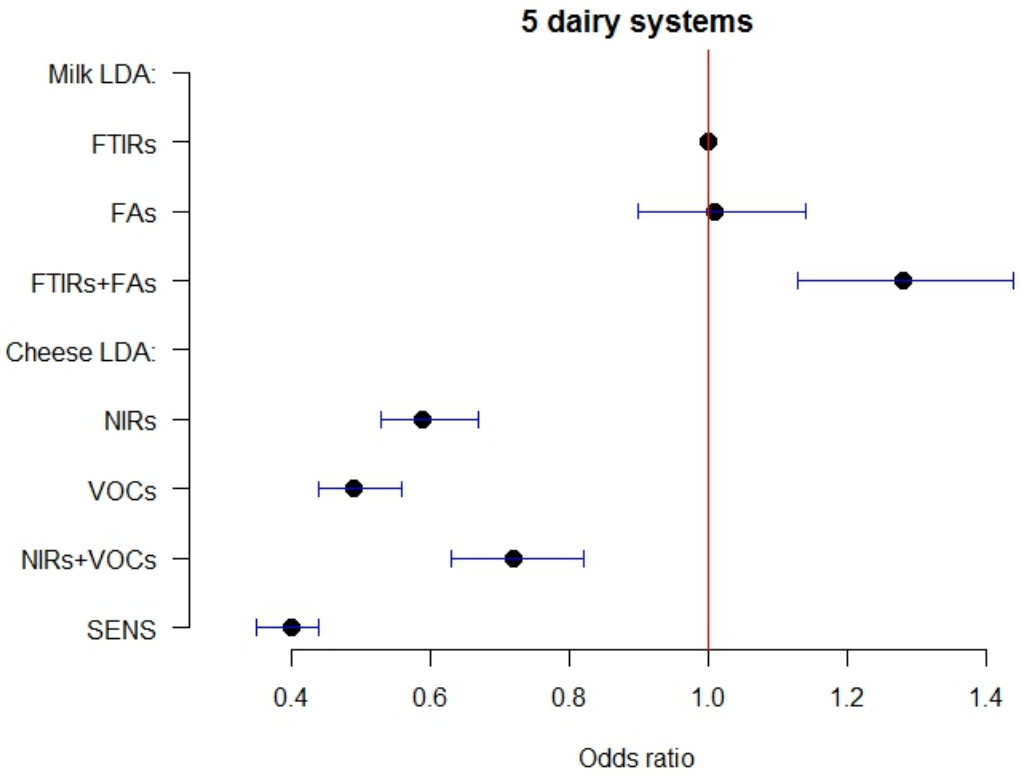
720 [a]The odds ratio in bold are characterized by 95% credibility regions not including 1.00 and
721 then considered superior (>1.00) or inferior (<1.00) respect to reference (FTIRs); [b] = reference
722 method. [c]AF = automatic feeder; [d]TMR = total mixer ration.
723

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

729

730 ***Caption***

731 **Figure 1.**

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

735

736 *Supplementary materials*

737 **Supplementary Figure S1.**

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

742 **Supplementary Figure S2.**

743 Figure S1. Principal component analysis (PCA) of NIRS spectra measured from 904 cheese sample
744 originated from milk sample collected in dairy farms classified as traditional farms, modern farms
745 without the use of silages, and modern farms using silages. Components 1 and 2 explain 70.29 and
746 24.97% of the variance, respectively.

747