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Infrared spectroscopic methods for the discrimination of cows' milk according to the feeding system, cow breed and altitude of the dairy farm

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A B S T R A C T

Bulk milk samples were collected from four French regions to study the potential capability of mid-infrared (MIR) and near-infrared (NIR) spectroscopy data to differentiate milk according to the feeding system, breed of cow and altitude of the farm. The MIR method demonstrated an excellent capability to distinguish milk from hay- and pasture-based systems and those from maize silage- and pasture-based systems. The MIR method did not exhibit the same capability concerning the discrimination of milk from hay- and maize silage-based systems. A similar trend was observed with the NIR method but with lower efficiency. The two infrared methods did not satisfactorily discriminate milk from different cow breeds. Significant differences ($P < 0.05$) between methods in the proportion of correctly classified samples according to the feeding system and breed were reported, whereas no significant differences were found between the methods concerning the discrimination of lowland versus upland samples.

1. Introduction

Food crisis in recent decades have reduced consumer confidence in the food industry and increased the need for accurate information concerning different steps in the food chain. Increasing consumer sensitivity to food processing has progressively created a new concept of quality, particularly for animal products, which couples traditional indicators (sensorial, colour and nutritional properties) with new indicators concerning ethical aspects, such as animal welfare and environmental impact (Luykx & van Ruth, 2008). Indeed, consumers consider that a cows' diet based on pasture to be more natural and respectful to animal welfare. Low-input systems are considered to be more sustainable than intensive systems, which have been accused of releasing high amounts of negatively-perceived products, such as nitrogen, and having a high ecological footprint. For these reasons, the interest of consumers has led to a focus on tracing animal feed at the moment of the purchase decision. The European Union recognised the importance of the consumers' requests, and promulgated the General Food Law (European Commission, 2003) that became effective in 2005 and made traceability compulsory for all feed businesses.

To date, several milk biomarkers, such as fatty acids (FAs), volatile compounds, vitamins, and isotopes have been proposed to identify the diets eaten by cows (Martin et al., 2005). Additionally, the altitude of the farms (Engel et al., 2007) and a wide variety of physico-chemical analyses have been used for this purpose (Ferlay et al., 2008; Fernandez, Astier, Rock, Coulon, & Berdagué, 2003;

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Renou et al., 2004; Vasta, Ratel, & Engel, 2007). There is no doubt about the effectiveness of these analyses, especially if they are used as integrated techniques (Engel et al., 2007). Nevertheless, these techniques are expensive, time consuming and/or require the presence of highly-skilled personnel, therefore it is not possible to use these techniques as tools for routine analyses.

Infrared (IR) spectroscopy represents a rapid, cheap and easy-to-use technique available both for research and for on-line and off-line analyses in food industries (Woodcock, Fagan, O'Donnell, & Downey, 2008). IR spectroscopy measures the fundamental vibrations of molecules, and each functional group has specific vibrational frequencies. Collectively, the effects of all of the different functional groups result in a spectrum representing a unique molecular 'fingerprint' that can be used for quantitative or qualitative purposes (Luykx & van Ruth, 2008). Several studies report on the use of mid-infrared (MIR; approx. 400-4000 cm^{-1}) and near-infrared (NIR; approx. 14,000–4000 cm^{-1}) absorbance spectra to determine the composition and nutritional properties of milk (Coppa et al., 2010; De Marchi et al., 2009; Soyeurt et al., 2006; Tsenkova, Atanassova, Itoh, Ozaki, & Toyoda, 2000), the chemical composition (Karoui et al., 2006; Lucas, Andueza, Ferlay, & Martin, 2008) and the geographical origin of these dairy products (Karoui et al., 2004). NIR spectroscopy has also been used to discriminate between pasture-fed and concentrate-fed lamb carcasses (Dian, Andueza, Jestin, Prado, & Prache, 2008) and to authenticate the cheese production chain (Cozzi, Ferlito, Pasini, Contiero, & Gottardo, 2009).

To our knowledge, there is a lack of studies on the feasibility of MIR and NIR spectroscopy methods to identify the feeding background of farm animals directly from non-experimental milk spectra. Therefore, the objective of this study was to investigate the potential use of MIR and/or NIR spectra to differentiate milk on the basis of the different diets given to the cows, the breed of cows and the altitude of the farms.

2. Materials and methods

2.1. Milk samples

The study was conducted for dairy cattle milk in 13 French departments located in four regions during 2010. Four different laboratories of the French Milk Recording Organisation (FMRO) were in charge of the milk sampling and analyses from specific departments based on their proximity to the farms. Bulk milk samples (986) representative of summer and winter feeding were collected: 92 samples were obtained from the department of Jura in the Franche-Comté region; 245 samples were obtained from the departments of Calvados, Manche, and Orne in the Normandy region; 285 samples were obtained from the departments of Loire-Atlantique, Maine-et-Loire, Mayenne, and Sarthe in the region of Pays de la Loire; and 364 samples were obtained from the departments of Côte d'Or, Doubs, Bas-Rhin, Haut-Rhin, and Territoire de Belfort in the regions of Burgundy, Alsace, and Franche-Comté (eastern France). Each sample, corresponding to two or four consecutive milkings, was subdivided into two subsamples in which bronopol was added as a preservative. One set of subsamples underwent MIR analysis in the same laboratory where it was collected to predict the protein and fat contents (AOAC, 1997) and to collect the spectrum. The second set of subsamples were frozen at −18 °C and sent to a laboratory for NIR spectroscopy analysis (INRA, UMR 1213 Herbivores, Theix, France).

2.2. Feeding systems

Two surveys were conducted by the FMRO on each farm to characterise the milk production conditions at the moment of sampling. Descriptive analyses of data were carried out on milk yield and composition, average breed composition for the herd of farm studied, altitude of the farms, type of feedstuff (pasture, hay, grass silage, maize silage, or concentrate) and the estimated percentage of each feedstuff given to the herd.

Ten variables (milk fat and protein contents, percentage of pasture, hay, grass silage, maize silage and concentrate in the ration (on a dry matter, DM, basis), and percentage of Holstein, Montbéliarde and Normande cows present in each farm) were used in a principal components analysis (PCA; Minitab, State College PA, USA). This analysis allowed us to distinguish three main feeding systems defined by the dominant forage: maize silage, pasture and hay (data not shown).

Among a total of 986 milk samples analysed, we selected 676 samples in which the dominant forage in the diet represented a minimum of 65% of total diet on a DM basis (Esvan et al., 2010). The selected samples were further assigned to four groups on the basis of the geographical distribution and laboratory of collection as follows: group A contained 79 samples from the Jura department; group B contained 253 samples from the Burgundy, Alsace, and Franche-Comté regions (eastern France); group C contained 170 samples from the Normandy region and group D contained 174 samples from the regions of Pays de la Loire and Brittany. The lowland and upland groups were distinguished according to the altitude. The samples were considered to belong to the lowland and upland groups when the altitude of the farm was lower than 600 m and higher than 700 m, respectively (Engel et al., 2007). The samples were also pooled according to the breeds reared in the farms: milk samples were assigned to the Montbéliarde, Normande, or Holstein group when at least 80% of the reared cows in the farm belonged to the same breed.

2.3. Reflectance spectra in the near-infrared spectroscopy

All of the selected samples were analysed in the same laboratory, as reported by Coppa et al. (2010). Briefly, after 2 h at room temperature, milk samples (0.5 mL) were placed on a glass microfibre filter (Whatman GF/A, 55 mm; Whatman International Ltd, Maidstone, UK) and oven-dried at 40 °C for 24 h. The samples were then placed in a 50 mm-diameter ring cup and scanned in reflectance mode at 2 nm intervals from 1100 to 2498 nm using a 6500 NIR scanning spectrometer (Foss NIRSystems, Silver Spring, MD, USA) equipped with an autocup module and controlled via ISIscan software version 2.21 (Infrasoft International LLC, State College, PA, USA). Each reflectance spectrum was time-averaged from 32 scans and was compared with the 32 average-measurements of a ceramic reference.

2.4. Absorbance spectra in the mid-infrared spectroscopy

Fresh milk samples were also analysed using four Milkoscan FT6000 MIR spectrometers (Foss, Hillerød, Denmark) located in different laboratories of the FMRO in charge of the collection of milk samples. The MIR spectra were recorded from 5012 to 926 cm^{-1} with a spectral resolution of 3.85 cm^{-1}. The samples were extracted using FossLims Integrator software (Foss), and then forwarded to the PhénoFinlait database using ExportDataLab software (Institut de l'Elevage, Paris, France).

2.5. Methods used to distinguish feeding systems

Because the MIR spectra were collected in different laboratories by different instruments, and in the absence of milk control samples analysed by these laboratories in order to standardize the MIR spectra, it was not possible to use these spectra as a unique dataset. The potential of MIR and NIR spectroscopy to discriminate milk samples according to the diets fed to the dairy cows, the cow breeds or the altitude of the farms was studied within each laboratory. Therefore, the spectra from group A were used to distinguish between the feeding systems based on pasture versus hay, and the samples from group B were used to discriminate among the three feeding systems. Moreover, because different farm altitudes were found in group B, the samples from this group were also used to discriminate between the lowland and upland systems. Group C and D were independently used to discriminate between systems based on maize silage and pasture. The samples from group B were used to distinguish between Montbéliarde and Holstein cows, whereas the samples from groups C and D were independently used to distinguish between Normande and Holstein cows.

In the NIR spectroscopy method, the standard normal variate and de-trend (SNVD) scatter correction procedure (Barnes, Dhanoa, & Lister, 1989) was applied to the raw data. The spectra were then transformed using a mathematical first-order gap derivation (1,4,4,1), in which the first digit is the order of the derivative, the second digit is the gap over which the derivative is calculated, the third digit is the number of data points in the first smoothing, and the fourth digit is the number of data points in the second smoothing. These mathematical transformations allowed the best results in the different comparisons. For each group of milk samples, the derivative spectra underwent a partial least squares discriminant analysis (PLS-DA) to differentiate between the two feeding treatments, the three cow breeds, or the two groups according to the altitude of the farms. PLS-DA is a regression in which the dependent variable is a set of categorical variables describing the different classes of observations. Each sample is assigned a dummy variable. The model was tested using a cross validation procedure in which randomly selected samples (25% of the total samples) were temporarily removed from the initial data set to be used for validation. This procedure was repeated four times until all data set samples had been used in the validation procedure. The cross-validation error was then calculated. The analyses were carried out using WinISI III v 1.6 software (Infrasoft International LLC).

In the MIR spectroscopy method, the raw spectra were transformed using a mathematical first-order gap derivation (1,4,4,1), in which the first digit is the order of the derivative, the second digit is the gap over which the derivative is calculated, the third digit is the number of data points in the first smoothing, and the fourth digit is the number of data points in the second smoothing. The derivative spectra underwent the same PLS-DA and cross-validation procedure described above for the NIR spectra.

2.6. Statistical analysis

The proportion of correctly classified milk samples was analysed according to the following method:

$$Y = \mu + Si + \varepsilon ijk \quad (1)$$

where Y = dependent variable, μ = overall mean, S = spectral mode effect (1 df), ε = experimental error. The analyses were carried out using the CATMOD procedure of SAS/STAT software (version 9.1; SAS Institute Inc., Cary, NC, USA) using the spectral mode with repeated measures as a factor. The bulk milk collected at each farm was taken as the statistical unit.

Table 1
Diet composition for the different production systems.[a]

Parameter	Maize silage	Pasture	Hay	Maize silage	Pasture	Hay
	Group A			*Group B*		
n	–	31	48	34	124	95
Pasture (%)	–	84.7 (68.1–100)	0	21.1 (0–43.4)	85.7 (65.83–100)	0
Maize silage (%)	–	0.4 (0–12.4)	0	73.0 (65.2–100)	2.1 (0–30.5)	0
Hay (%)	–	7.9 (0–29.6)	92.1 (72.5–100)	1.5 (0–21.4)	8.9 (0–28.8)	93.4 (66.2–100)
Grass silage (%)	–	0.6 (0–16.6)	0	0.7 (0–16.1)	0.2 (0–17.4)	0.7 (0–33.1)
Concentrate (%)	–	6.4 (0–22.6)	7.83 (0–27.5)	2.8 (0–12.4)	2.9 (0–17.7)	5.5 (0–26.8)
Other (%)	–	0	0.1 (0–3.6)	1.0 (0–21.4)	0.2 (0–18.9)	0.5 (0–19.4)
Montbéliarde (%)	–	94 (64–100)	97 (75–100)	63 (0–100)	93 (0–100)	97 (0–100)
Normande (%)	–	0	0	0	0	0
Holstein (%)	–	0	0.1 (0–5)	36 (0–100)	5.2 (0–100)	3 (0–97)
Milk yield (kg per day)	–	23.5 (17.0–30.0)	23.5 (16.0–32.0)	25.3 (20–33)	24.9 (13.0–32.0)	23.9 (12.0–32.0)
Lactation rank	–	2.75 (2–4)	2.89 (2–4)	2.71 (2–3)	2.74 (2–4)	2.69 (2–4)
Lactation stage (days)	–	193 (134–253)	135 (98–184)	157 (91–305)	172 (100–230)	131 (77–205)
No. lactating cows (per farm)	–	50.3 (24–76)	50.2 (21–94)	61.8 (13–114)	45.6 (19–135)	43.2 (12–121)
Altitude (m)	–	506 (50–950)	500 (50–1100)	288 (100–480)	648 (100–1100)	687 (100–1100)
	Group C			*Group D*		
n	116	54	–	111	63	–
Pasture (%)	0.5 (0–32.9)	77.7 (65.4–100)	–	1.2 (0–34.2)	79.5 (65.2–100)	–
Maize silage (%)	79.4 (65.2–97.5)	17.8 (0–34.0)	–	74 (65–100)	16.9 (0–31.5)	–
Hay (%)	3.4 (0–17.9)	1.9 (0–21.3)	–	3.3 (0–15.4)	1.6 (0–13.2)	–
Grass silage (%)	7.2 (0–33.1)	0.5 (0–17.6)	–	9.3 (0–30.9)	0.03 (0–2.3)	–
Concentrate (%)	7.5 (0–20.5)	1.4 (0–21.3)	–	7.8 (0–23.0)	1.5 (0–13.3)	–
Other (%)	2.7 (0–19.5)	0.7 (0–32.6)	–	0.9 (0–23.0)	0.4 (0–21.4)	–
Montbéliarde (%)	2 (0–93)	0 (0–2)	–	5 (0–98)	8 (0–98)	–
Normande (%)	60 (0–100)	74 (0–100)	–	41 (0–100)	49 (0–100)	–
Holstein (%)	36 (0–100)	25 (0–100)	–	53 (0–100)	43 (0–100)	–
Milk yield (kg per day)	22.53 (15.0–35.0)	21.9 (14.0–30.0)	–	23.4 (15.0–33.0)	23.0 (13.0–32.0)	–
Lactation rank	2.47 (2–3)	2.48 (2–3)	–	2.33 (2–3)	2.48 (2–4)	–
Lactation stage (days)	145 (69–227)	187 (122–284)	–	161 (99–257)	199 (125–296)	–
No. lactating cows (per farm)	61.6 (23–163)	58.9 (25–110)	–	62.6 (30–168)	61.6 (28–165)	–
Altitude (m)	150 (70–300)	146 (70–390)	–	118 (60–330)	130 (70–320)	–

[a] Results are expressed as means, and maximum and minimum values are in brackets; n: number of samples. Forages are expressed as % of total diet dry matter; *n*, number of samples.

3. Results

3.1. Comparison between the feeding systems

Table 1 reports the diet composition of the three feeding systems and the number of farms assigned to each system on the basis of the dominant forage in the total diet DM (minimum 65% of DM). The percentages of pasture and maize silage in the total diet were higher and lower for groups A and B than for groups C and D, respectively. The maize silage-based systems were similar between groups C and D whereas group D had a higher pasture percentage. No remarkable differences were reported between the hay-based systems of group A and B.

3.2. Discrimination of feeding systems by the MIR spectroscopy method

For the comparison between the pasture- and hay-based systems, the PLS-DA models (Table 2) allowed for the correct classification of 96.2 and 98.2% of the milk samples from the groups A and B, respectively. Regarding the discrimination of the milk samples between the pasture and maize silage systems, 99.4, 98.8, and 94.8% of the milk samples from groups B, C and D were correctly assigned, respectively. The cross-validation error accounted for 14.7% of the samples when the MIR spectra were used to discriminate the maize silage from the hay systems in group B.

3.3. Discrimination of feeding systems by the NIR spectroscopy method

The PLS-DA analysis (Table 2) allowed for the correct classification of 89.9 and 75.3% of the samples belonging to the pasture and hay systems of groups A and B, respectively. Regarding the discrimination between the pasture and maize silage systems, 89.8, 93.6, and 89.7% of the milk samples from groups B, C and D, respectively, were correctly assigned. The cross-validation error accounted for 22.6% of the samples when the NIR spectra were used to discriminate the maize silage from the hay systems in group B.

3.4. Discrimination between lowland and upland systems

The differences in the altitude of the farms were recorded for group B. The mean diet composition of the lowland and upland groups is reported in Table 3. For the upland group, 96.4% of the total diet DM was represented by pasture and hay whereas 36% of the total diet DM was maize silage in the lowland group.

Table 4 reports the comparison of the spectroscopic methods used to discriminate between the lowland and upland groups. The NIR and MIR spectroscopy methods allowed for the correct classification of 71.5 and 74.6% of the samples, respectively. Taking into account the differences in the diet composition between the two systems, we also tried to discriminate between altitudes under similar feeding conditions. Therefore, milk samples obtained from farms that had maize silage in the diet were excluded from the PLS-DA analysis. The results show that regardless of the feeding conditions, neither the MIR spectroscopy method nor the NIR spectroscopy method is suitable to discriminate between the upland and lowland groups (Table 4).

Table 3
Diet composition according to altitude (Group B).[a]

Parameter	Production systems	
	Lowland	Upland
n	223	124
Altitude (m)	309 (100–600)	825.5 (700–1100)
Pasture (%)	23.1 (0–100)	48.5 (0–100)
Maize silage (%)	35.6 (0–91.96)	0
Hay (%)	23.8 (0–100)	47.9 (0–100)
Grass silage (%)	7.8 (0–53.1)	0
Concentrate (%)	7.6 (0–35)	3.4 (0–25.0)
Other (%)	2.2 (0–74.54)	0.2 (0–14.9)
Montbéliarde (%)	66 (0–100)	91 (0–100)
Normande (%)	0	0
Holstein (%)	29.9 (0–100)	7 (0–100)
Milk yield (kg per day)	25.1 (12–35)	25 (19–34)

[a] Lowland: altitude <600 m; upland: altitude > 700 m. Forages are expressed as % of total diet dry matter. The results are expressed as means, and the maximum and minimum values are in brackets; *n*, number of samples.

Table 4
Percentage of cross validation error in the discrimination of milk samples according to altitude using the mid-infrared (MIR) and near-infrared (NIR) spectroscopy methods.[a]

Maize silage in the lowland diet	*n*	MIR		NIR		*P*
		Correctly classified	% Error	Correctly classified	% Error	
Yes	347	248	28.5	258	25.6	0.31
No	199	131	34.2	134	32.7	0.70

n: number of samples.
[a] Lowland: altitude <600 m; upland: altitude > 700 m.

3.5. Discrimination between reared breeds

Table 5 shows the diet composition of the different groups used for the discrimination between breeds. Table 6 reports on the performance and the comparison of the spectroscopic methods used to distinguish between milk samples of the breeds in the different groups. The MIR and NIR spectroscopy methods allowed for the correct classification of 85.3 and 87.5% of milk samples from the Montbéliarde and Holstein cows in group B, respectively. Regarding the discrimination between the Normande and Holstein cows, the MIR and NIR spectroscopy methods allowed for a correct assignment of 89.9 and 75.3% of the milk samples from group C, respectively, and of 89.1 and 68.8% for those in group D, respectively.

3.6. Comparison of the spectroscopic methods used

Regarding the discrimination between the feeding systems, the proportion of correctly classified samples significantly differed

Table 2
Percentage of cross validation error in the discrimination of milk samples according to feeding system using the mid-infrared (MIR) and near-infrared (NIR) spectroscopy methods.[a]

Group	Pasture versus hay				Pasture versus maize silage				Maize silage versus hay			
	n	MIR %error	NIR %error	*P*	*n*	MIR %error	NIR %error	*P*	*n*	MIR %error	NIR % error	*P*
A	79	3.80	10.13	0.07								
B	219	1.83	24.66	<0.001	158	0.63	10.19	<0.001	129	14.7	22.6	0.05
C					170	1.18	6.47	0.09				
D					174	5.17	10.34	0.39				

[a] *n*, number of samples.

Table 5
Diet composition for the different breeds in the different groups.[a]

Parameter	Group B		Group C		Group D	
	Montbéliarde	Holstein	Normande	Holstein	Normande	Holstein
n	271	56	115	43	87	105
Pasture (%)	39.8 (0–100)	11.2 (0–90.7)	29.3 (0–100)	21.5 (0–100)	32.48 (0–100)	22.9 (0–100)
Corn silage (%)	13.42 (0–92.0)	43.7 (0–84.2)	46.4 (0–94.3)	60.7 (0–97.1)	48.5 (0–100)	52.4 (0–97.4)
Hay (%)	10.31 (0–100)	15.9 (0–92.4)	7.8 (0–100)	2.9 (0–19.2)	3.5 (0–39.7)	3.2 (0–64.3)
Grass silage (%)	2.4 (0–35.7)	14.4 (0–53.1)	7.6 (0–100)	4.7 (0–28.7)	9.6 (0–55.1)	12.7 (0–71.2)
Concentrate (%)	4.9 (0–35.0)	9.8 (0–26.3)	5.4 (0–20.9)	6.7 (0–20.35)	5.6 (0–17.2)	7.49 (0–23.6)
Other (%)	0.6 (0–26.0)	4.9 (0–74.5)	3.5 (0–81.4)	3.5 (0–81.5)	0.3 (0–12.6)	2.1 (0–90.5)
Milk yield (kg per day)	24.7 (16.0–33.0)	26.1 (15.0–35.0)	20.6 (12.0–29.0)	27.5 (19.0–41.0)	20.9 (13.0–27.0)	26.2(15.0–33.0)
Altitude (m)	573 (100–1100)	253 (100–650)	150 (70–280)	170 (70–390)	115 (60–330)	132 (70–320)

[a] The Normande breed was not represented in Group B, the Montbéliarde breed was not represented in Groups C and D. Forages are expressed as % of total diet dry matter. The results are expressed as means, and the maximum and minimum values are in brackets. The number of cows belonging to the same breed is up to 80%; *n*, number of samples.

Table 6
Percentage of cross validation error in the discrimination of milk samples according to the breed using the mid-infrared (MIR) and near-infrared (NIR) methods.

Group	*n*	Montbéliard versus Holstein					Normande versus Holstein				
		MIR		NIR		*P*	MIR		NIR		*P*
		Correctly classified	% Error	Correctly classified	% Error		Correctly classified	% Error	Correctly classified	% Error	
B	327	279	14.7	286	12.5	0.25					
C	158						142	10.1	109	24.7	0.041
D	192						171	10.9	134	30.2	0.005

n: number of samples.

($P < 0.05$) or tended to differ between the methods for all of the comparisons, except for the comparison of pasture versus maize silage in group D (Table 2). The MIR and NIR spectroscopy methods did not significantly differ with respect to the discrimination of the lowland and upland groups. Additionally, there was no significant difference when the milk samples from the lowland and upland groups were compared under similar feeding conditions (Table 4). The two methods significantly differed with respect to the discrimination of milk samples from the Normande versus Holstein breeds, and no significant difference was found for the discrimination between the milk samples from the Montbéliarde versus Holstein cows (Table 6).

4. Discussion

The feeding systems identified by PCA correspond to the main feeding systems existing in France. Specifically, the pasture-based system is the typical feeding system during spring and summer seasons both in the north-west and southern regions of France. Moreover, because a mountain is present in the eastern France and Franche-Comté region, access to the pasture is limited in the winter. The maize silage-based system is the main feeding system used in the northern region, whereas in the departments of the southern region involved in our study, grazed grass was replaced by hay in the diet during the winter.

The MIR spectroscopy method demonstrated an excellent capability to distinguish between milk samples from the hay and pasture systems and between the maize silage and pasture systems. The MIR spectroscopy method did not exhibit the same capability concerning the discrimination of milk samples from the hay- and maize silage-based systems. An identical trend was recorded for the NIR spectroscopy method, which distinguished pasture from hay samples and pasture from maize silage samples with higher efficiency, but this method exhibited a lower efficiency of discrimination between the samples of the maize silage- and hay-based systems.

The impact of cows' diet on milk composition is important, whereas lactation stage and parity have minor effects (Kelsey, Corl, Collier, & Bauman, 2003). In particular, the nature of the forage, and the forage-to-concentrate ratio influence the fat content and the fatty acid composition of milk (Chilliard et al., 2007), and the milk protein content can be influenced by dietary energy (Walker, Dunshea, & Doyle, 2004). The effect of the diet on milk components has been used to propose a set of biomarkers (Martin et al., 2005), reflecting the type of cow feeding and/or production zone. Natural pigments, such as lutein, β-carotenes and zeaxanthin in forages have been proposed as tracers for diets based on grazed grass, hay or maize silage because the forage content and subsequent transfer into milk vary according to the type of forage, the storage duration and the conservation mode (Calderón et al., 2007). An increase in the milk content of polyunsaturated FAs (PUFAs) and odd- and branched-chain FAs (OBCFAs) and a decrease in the content of saturated FAs are concomitant with the presence of grazed grass in the diet (Chilliard et al., 2007; Ferlay et al., 2008). Even if the concentration of PUFAs and/or OBCFAs in milk can be altered by the addition of oilseeds rich in PUFAs (Chilliard et al., 2007), these FAs could be considered good markers of a diet based on grazed grass (Engel et al., 2007). The presence of volatile compounds in milk and dairy products can be affected by animal feeding. These compounds can arise from a direct transfer from the feed, or they can be endogenously metabolised by the animal or ruminal microorganisms (Coppa et al., 2011; Vasta & Priolo, 2006).

In addition to their use to predict milk constituents and gross composition (Coppa et al., 2010; Karoui & De Baerdemaeker, 2007; Karoui et al., 2006), the two infrared methods used in this study are techniques that reflect the chemical characteristics of a specific milk via absorbance spectra. The differences in the milk composition arising from the feeding practices were likely detected in the spectra, allowing for the discrimination between pasture- and maize silage-based systems. Our results are in line with literature reports describing the possibility of using spectral methods to trace feeding systems. Using visible and NIR spectroscopy analyses of bulk milk samples, Martin, Jestin, Constant, Agabriel, and Andueza (2006) and Nozière et al. (2006) reported on the discrimination between milk samples from grazing cows and those from cows fed maize silage. These authors also discriminated between milk samples from cows fed hay and milk samples from those fed grass. Moreover, milk samples from organic and conventional systems were satisfactory discriminated by the milk content of omega-3 FAs predicted by NIR spectroscopy (Aulrich & Molkentin, 2009). Mouazen, Dribi, Roissi, De Baerdemaeker, and Ramonof (2009) achieved a successful discrimination between milk samples from pasture-fed ewes and those provided with an indoor diet using the milk composition predicted by the visible and NIR spectra. Likewise, studies on meat (Dian et al., 2008) and cheese (Cozzi et al., 2009) confirmed the possibility of using NIR spectroscopy to discriminate between pasture- and hay-based systems and between pasture- and maize silage-based systems in matrices different from milk. To our knowledge, there is no information on the capacity of MIR and NIR spectroscopy methods to discriminate between milk samples obtained from hay-based systems from those obtained from maize silage-based systems. However, similar to Martin et al. (2005), who reported the difficulty of discriminating milk samples obtained from hay and maize silage feeding by the use of terpenes as biomarkers, the infrared methods used herein did not allow for a satisfactory distinction between these two feeding systems. Similar results were obtained by Cozzi et al. (2009) using colour and chemical parameters of cheeses (moisture, total fat and protein content, and water-soluble nitrogen) as discriminating factors.

Even when the differences were significant, no large differences were recorded concerning the capacity of the two methods to discriminate between the lowland and upland groups. There were also no large differences observed when the two methods were used to discriminate between milk samples from the lowland and upland groups under similar feeding conditions. Engel et al. (2007) demonstrated the capacity of molecular biomarkers (fatty acids, vitamins, and carotenoids) to distinguish between milk samples obtained from the lowland (maize silage) and upland (pasture) regions. Moreover, Cozzi et al. (2009) found that cheeses produced at different altitudes can be distinguished by NIR alone when the samples represent real diverse feeding systems (maize silage- versus pasture-based systems). Our results indicate that neither the MIR spectroscopy method nor the NIR spectroscopy method is suitable for discriminating between milk samples from the lowland and upland groups. Under similar feeding conditions, NIR spectroscopy did not discriminate between milk samples arising from farms located at different altitudes (Coppa et al., 2012).

Under our conditions, it was not possible to use the NIR or MIR spectroscopy methods to successfully discriminate among samples from the different cow breeds. This result is consistent with the inability to discriminate between sheep genotypes reported by Mouazen et al. (2009). Because high differences in the diet consumed by the cows of the three breeds were recorded in our study (Table 5), we can suggest that the diet effect on the milk composition was stronger than the breed effect, as previously reported in the literature (Ferlay et al., 2010; Palmquist, Beaulieu, & Barbano, 1993). Therefore it was not possible to discriminate among the milk samples from different breeds.

The MIR spectroscopy method exhibited a significantly greater efficiency in the discrimination of feeding systems than the NIR method. In previous studies, the MIR and NIR spectroscopy methods have been compared to examine their capability to predict selected chemical parameters or to determine the geographical origin of cheese, oil and other products such as honey and wine. For example, McQueen, Wilson, Kinnunen, and Jensen (1995) compared the NIR and MIR spectroscopic methods to predict the protein, fat, and moisture contents of cheese samples and concluded that NIR spectroscopy was a more attractive technique in terms of prediction precision. Moreover, Karoui et al. (2006) compared the two methods for the prediction of the chemical parameters of cheeses from different geographical zones and concluded that a higher precision in the predictions of the contents of fat, DM, total nitrogen and water soluble carbohydrates was obtained with visible-NIR spectroscopy than with the MIR spectroscopy method. These results suggest a higher capability of the NIR spectroscopy method to discriminate between feeding zones. The bronopol that was used as a preservative in our study at different and unknown concentrations may have influenced the infrared spectra. Indeed, the presence of bronopol was demonstrated to affect the determination of milk components by MIR spectroscopy because the CH_2 groups in the structure of bronopol absorb light at the CH stretch wavelengths, which are identical to those of fat, protein and lactose (Barbano, Wojciechowski, & Lynch, 2010). Further research is needed to analyse the effect of the presence of bronopol on the results obtained with the two methods to clarify whether the NIR spectroscopy data are more influenced by bronopol than the MIR spectroscopy data.

5. Conclusions

The MIR and NIR spectroscopies are capable of distinguishing between milk samples from different feeding systems, particularly when hay from pasture systems and maize silage from pasture systems were compared. To the best of our knowledge, this is the first study to analyse the ability of the MIR and NIR spectroscopies to discriminate between feeding systems under real husbandry conditions. Our results suggest that the MIR spectroscopy method is more efficient than the NIR spectroscopy method for this purpose.

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