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Mid infrared attenuated total reflection spectroscopy as a rapid tool to assess the quality of Sicilo-Sarde ewe's milk during the lactation period after replacing soybean meal with scotch bean in the feed ration

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Abstract

This study investigates the potential of attenuated total reflection spectroscopy in the mid infrared (MIR) for monitoring changes in the quality of ewe's milk as a function of lactation period and feeding systems. Twelve 5-year-old lactating Sicilo-Sarde ewes (third lambing) were kept in environmentally controlled sheepfolds and were divided into two homogenous weight matched groups ($n = 6$). Ewes were fed *ad libitum* with two *iso*-energetic diets (20% barley, 3% vitamin and mineral premix, and 77% soybean meal or scotch bean). Physico–chemical analyses and MIR (3000–900 cm^{-1}) were performed on milk samples after 1, 2, 3, 4, 5, 6, 7, 8, 9 and 10 weeks of lactation period. The inclusion of scotch bean in the diet resulted in a significant decrease ($P \leqslant 0.05$) of fat content (7.85 g 100 g^{-1} vs. 6.75 g 100 g^{-1}) and a significant increase ($P \leqslant 0.05$) of lactose level (3.49 g 100 g^{-1} vs. 3.61 g 100 g^{-1}). The principal component analysis (PCA) applied to the 1700–1500 cm^{-1} spectral region showed only some discrimination between milk samples according to diet compositions. The best results were obtained in the 3000–2800 cm^{-1} and 1500–900 cm^{-1} spectral regions since a good discrimination between milk from ewes fed soybean meal from those fed scotch bean meal was observed. It can be concluded that these spectral regions could be considered as fingerprint, regions allowing a good identification of milk according to diet composition. However, the MIR failed to discriminate milk samples according to the lactation period for the two feeding systems.

Keywords: Ewe's milk; Lactation; Soybean meal; Scotch bean; Mid infrared; Chemometric

1. Introduction

An interest in the production and processing of sheep milk has increased in the Republic of Tunisia in the last few years (Atti, Rouissi, & Othmane, 2006). Milk production is predominantly based on different breeds with local characteristics adapted to meagre vegetation, varied climatic conditions, shallow soil and lack of precipitation. The quantity, chemical composition, and physical properties of milk are influenced by several factors such as genetic (breed and genotype), physiological (age, lambing, body weight, stage and number of lactation), health and growth rate of ewes (Bencini & Paulina, 1997). Furthermore, the stage of lactation and feeding systems significantly influenced the chemical composition of ewe's milk (Atti et al., 2006; Buccioni et al., 2006; Fuertes, Gonzalo, Carriedo, & Sanprimitivo, 1998; Gonzalo, Carriedo, Baro, &

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Primitivo, 1993; Jelinek, Gajdušek, & Illek, 1993; Maria & Gabina, 1993; Peart, 1975).

The influence of the above mentioned factors on the quality of ewe's milk have, until now, been measured using popular and well-known analytical methods such as gas chromatography (Atti et al., 2006), liquid chromatography (Kráčmar et al., 1998) and physico–chemical analyses (Pavic, Antunac, Mioc, Ivankovic, & Havranek, 2002; Sahan, Say, & Kaçar, 2005). However, all these techniques are tedious and destructive, relatively expensive, time-consuming and require highly skilled operators. Recently, attention has focused on the development of non-invasive and non-destructive instrumental techniques such as infrared and front face fluorescence spectroscopies. Infrared spectroscopy expresses typical vibration modes of covalent bonds in molecules, and thus contains quantitative information about all the constituents that absorb infrared radiation. The potential of near infrared spectroscopy (NIR) for the determination of total phosphorus in sheep's milk has been demonstrated by Dogan, Sahin, and Ulgen (2005). Many overlapping signals were observed in the NIR, which give rise to a spectrum with broad peaks, making it difficult to interpret compared to the conventional mid infrared (MIR) spectrum.

Because each chemical compound in the milk contributes to the absorbance spectrum, the MIR spectrum of a milk sample contains information for the compounds which are present at levels >0.1% (w/w). At present, MIR spectroscopy is among the most preferred method for dairy products analysis. This is due to the fact that almost every chemical substance (apart from some salts and very simple chemical compounds) has its own distinctive spectrum. Only substances occurring in very low concentrations can be difficult to determine, as the noise level of the method might be encountered in such a case. The water absorbs in the MIR region and may affect the interpretation of the spectra. Water is a very strong infrared absorber with prominent bands centered at 3360 cm^{-1} (H–O stretching band), 2130 cm^{-1} (water association band) and 1640 cm^{-1} (the H–O–H bending vibration) (Safar, Bertrand, Devaux, & Genot, 1994). Precise subtractions of the H_2O bands is possible for pure solutions because of the frequency precision achievable with Fourier transform infrared. However, it is difficult to subtract precisely the water absorption in complex, emulsified and colloidal suspensions such as milk. The development of the attenuated total reflectance (ATR) device allows the sampling problems encountered when collecting spectra from opaque and viscous samples to be overcome. This technique has been used to determine the: (i) protein concentration (Etzion, Linker, Cogan, & Shmulevich, 2004) in raw cow's milk, (ii) nutritional parameters of commercially available milks (Iñón, Garrigues, & de la Guardia, 2004) and (iii) conjugated linoleic acids (CLA) in cow's milk (Meurens, Baeten, he Yan, Mignolet, & Lardondelle, 2005).

The potential of MIR to determine the quality of milk has been widely performed on cow's milk. However, to our knowledge, no study has so far assessed the potential of MIR to determine the quality of ewe's milk throughout the lactation period. Thus, the aim of the present study was to assess the potential of MIR coupled with chemometric tools to determine changes that occur in Sicilo-Sarde ewe's milk during lactation in accordance with the feeding of different diets: imported soybean meal vs. local produced scotch bean.

2. Materials and methods

2.1. Animals

Twelve 5-year-old Sicilo-Sarde ewes 42 ± 2 Kg, at their third lambing were kept at 17 °C in individual boxes and in environmentally controlled sheepfold under standard conditions to single boxes on a peat litter in order to control performances and intakes individually. Zootechnical performances and energy intake were recorded individually.

2.2. Diets

After 2 weeks of adaptation, ewes were divided into two homogenous weight matched groups ($n = 6$). The 2 experimental diets differed in terms of protein sources (soybean meal or scotch bean). Ewes remained under experiment until 10 weeks post-partum. Diets were *iso*-energetic and were given in restricted amounts according to the feed intake of the two groups.

2.3. Physico–chemical analyses

The ewes were milked one time per day (at 11:00 h). Milk samples of each group were gathered in a single box and analysed only one time per week for pH, density, non fat in dry matter, fat, protein, lactose, ash and freezing point by using LactoScan (Milkotronic Ltd., Serial No. 4696, Hungary).

2.4. Mid infrared measurements

Milk samples were shaken gently before filling the ATR cell. The MIR spectra were recorded between 3000 and 900 cm^{-1} at a resolution of 4 cm^{-1} with a Fourier transform spectrometer IFS 66V/S (Bruker, Belgium). To improve the signal-to-noise ratio, 250 scans were accumulated for each spectrum. The instrument was allowed to purge for 5 min with nitrogen gas prior to acquisition of the spectra to minimise the spectral noise due to atmospheric carbon dioxide and water vapor. The sample station was equipped with an overhead multiple attenuated total reflection (m-ATR) accessory that contains transfer optics, through which infrared radiation can be directed to a detachable ATR crystal. The m-ATR cell used as a sampling accessory has a reflection horizontal ATR (HATR, Pike Technologies, Madison, USA) crystal made of zinc Selenide (ZnSe) with an incidence angle of 45° and a number of reflections of 10.

The background spectrum was scanned at the beginning of the measurement by filling the ATR cell with Millipore Q-purified water and using the same instrumental conditions as that used during spectra acquisition. The same procedure was used for scanning blank spectra. Blanks were scanned before and after the analysis of each milk sample in order to be able to correct-non specific changes in the sample spectra and to evaluate the robustness of the cleaning procedure. After each measurement, the m-ATR crystal was thoroughly washed with ethanol and distilled water and then dried. All experiments were done in triplicate.

2.5. Mathematical analysis of data

In order to reduce scattering effects and to compare the samples, MIR spectra were normalised by reducing the area under each spectrum to a value of 1 according to Bertrand and Scotter (1992). Mainly the shift of the peak maximum and the peak width changes in the spectra were considered following this normalisation. The PCA was applied to the normalised spectra to investigate differences between the milk samples. The PCA transforms the original variables into new axes called principal components (PCs), which are orthogonal, so that the data sets presented on these axes are uncorrelated with each other (Jolliffe, 1986). Therefore, PCA expresses as much as possible the total variation in the data set in only a few PCs and each successively derived PC expresses decreasing amounts of the variance. This statistical multivariate treatment was used previously to observe similarities among different milk samples (Karoui et al., 2005), reducing the dimension to two or three PCs, while keeping most of the original information found in the data.

The PCA was carried out using MATLAB Software (Version 6, Release 12, The MathWorks, Belgium).

Table 1
Average of the physico–chemical composition of Sicilo-Sarde ewe's milk fed soybean meal and scotch bean during 10 weeks of lactation period

Compositional parameter	Ration	
	Soybean meal	Scotch bean
pH	6.77[a]	6.83[a]
Density (g cm^{-3})	1.031[a]	1.032[a]
Non fat in DM (g 100 g^{-1})	9.97[a]	10.06[a]
Fat (g 100 g^{-1})	7.85[a]	6.75[b]
Protein (g 100 g^{-1})	5.54[a]	5.51[a]
Lactose (g 100 g^{-1})	3.49[a]	3.61[b]
Ash (g 100 g^{-1})	0.91[a]	0.92[a]
Freezing point (°C)	−0.498[a]	−0.507[a]

DM: dry matter.
[a,b] Means values within a row sharing a common superscript do not differ significantly ($P \leqslant 0.05$); values presented are mean values for 6 milk samples.

3. Results and discussion

3.1. Physico–chemical analyses

The results for density, non fat dry matter, fat, protein, lactose and ash contents, and pH and freezing point values of milk samples produced from ewes fed soybean meal and scotch bean meal during lactation period were depicted in Table 1. No significant difference was observed between the two feeding systems on pH values throughout the lactation period (Table 1).

The highest values of pH being 6.91 and 6.94 after 3 weeks and the lowest 6.65 and 6.69 after 10 weeks post-

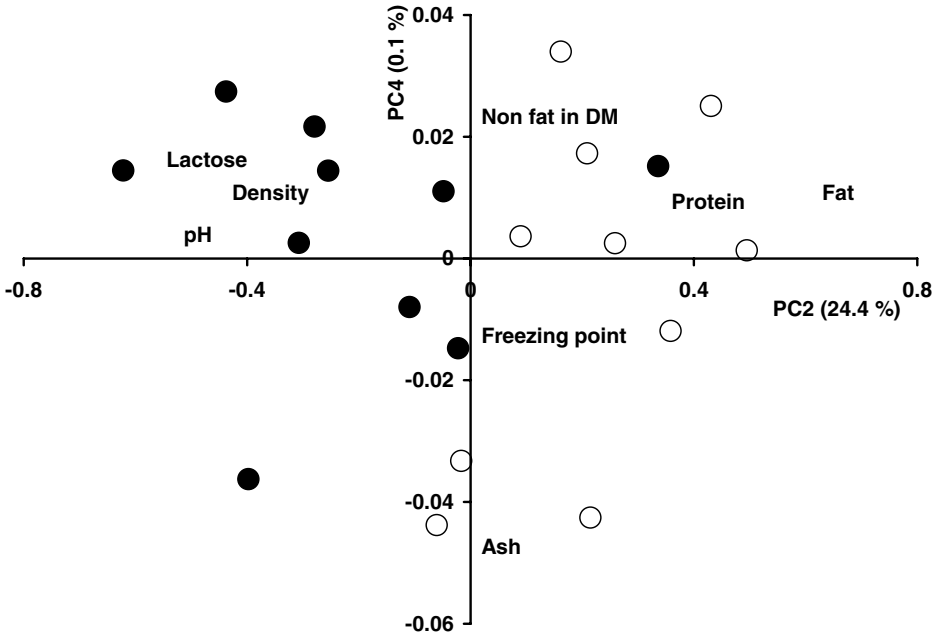

Fig. 1. Principal component analysis similarity map determined by principal components 2 (PC2) and 4 (PC4) of the physico–chemical parameters recorded on Sicilo-Sarde ewe's milk fed soybean meal (○) and scotch bean (●) during lactation period.

partum for ewe's fed soybean meal and scotch bean meal, respectively. For the two feeding systems, there was a significant decrease of pH values throughout the lactation period, in agreement with the findings of Shan et al. (2005).

Density, protein, and non fat DM levels were significantly ($P \leqslant 0.05$) lower at the beginning of the lactation period, whereas the freezing point values showed an opposite trend. The fat content of milk from ewes fed soybean meal and scotch bean meal decreased from the beginning of lactation until 6 and 5 weeks of lactation period, respectively; after that, the level of fat content increased continuously until the end of lactation. A similar variation pattern has been reported previously by Albenzio et al. (2004); these authors have observed an increase in the fat content of ewe's milk presenting low somatic cell count from 6.54 to 8.61 g 100 g^{-1} at early and late stage of lactation, respectively. In addition, the replacement of soybean meal by scotch bean meal induced a significant decrease ($P \leqslant 0.05$) in the level of lactose content as depicted in Table 1.

An increase in the values of protein content was observed throughout the lactation period, in agreement with the findings of Sahan et al. (2005). However, it seemed that the replacement of scotch bean by soybean meal did not significantly affect the protein content throughout all the lactation period considered in the present study (Table 1).

What ever the considered feeding system, an increase in the amount of lactose was observed throughout the lactation stage, which was pointed out by Antunović, Steiner, Senčić, Mandić, and Klapec (2001) during the lactation period of Würterberg breed ewes in both winter and summer seasons. Indeed, these researchers have observed an increase of the amount of lactose from 3.05 to

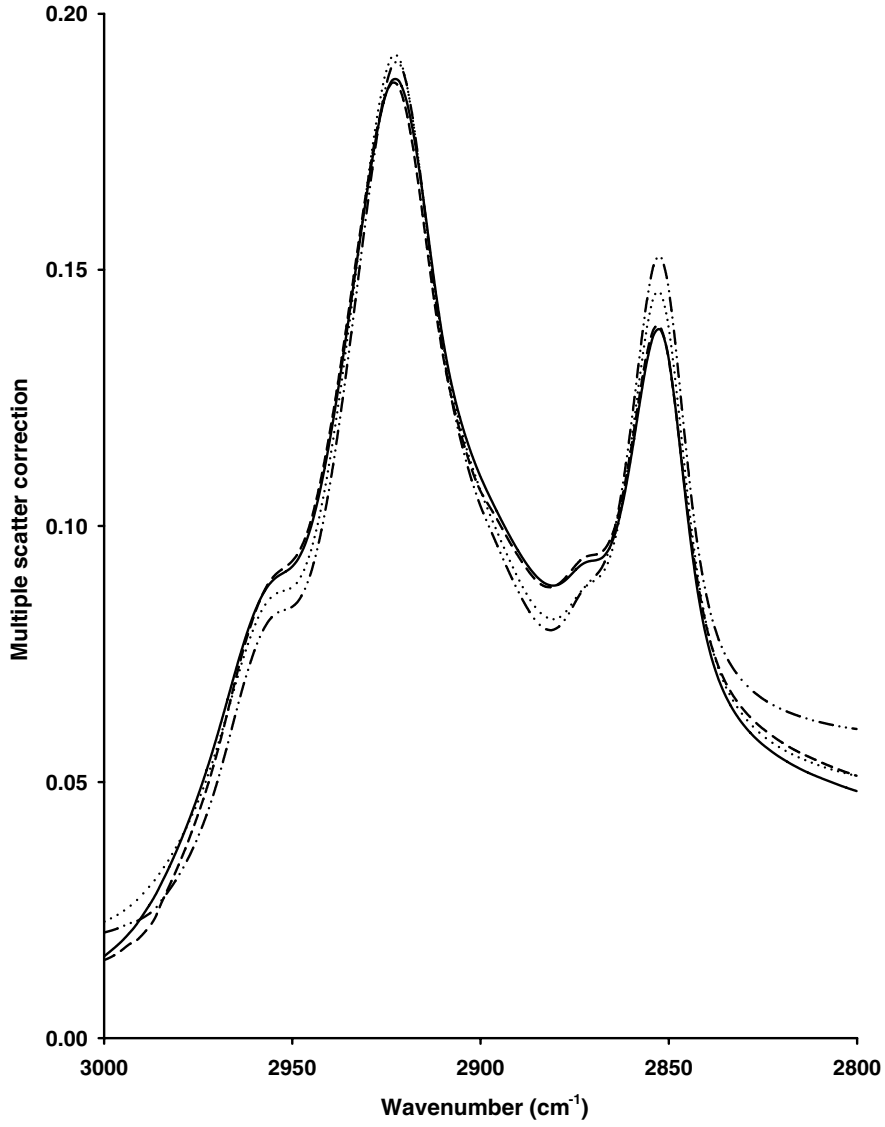

Fig. 2. Multiple scatter correction (MSC) applied to the 3000–2800 cm^{-1} spectral region recorded on Sicilo-Sarde ewe's milk fed scotch bean and soybean meals after 1 (—), (. . .), and 10 (– – –), (—..—..) weeks of lactation, respectively.

4.63 g 100 g^{-1} during winter season and from 3.2 to 4.91 g 100 g^{-1} during summer period, after 2 and 60 lactation days, respectively. In the present study, the replacement of soybean meal by scotch bean meal induced a significant increase ($P \leqslant 0.05$) in the level of lactose content as presented in Table 1. Considering the ash content, the lowest level being 0.87 g 100 g^{-1} and approximately 0.90 g 100 g^{-1} at the beginning of lactation period and the highest was 0.95 g 100 g^{-1} after 9 lactation weeks for ewes fed soybean meal and scotch bean meal, respectively.

In order to determine the relationship between physico and chemical parameters, PCA was applied to the normalised data sets. The obtained result is shown in Fig. 1. According to the principal component 2 (PC2) accounting for 24.4% of the total variance, milk from ewes fed scotch bean meal presented mostly negative scores, while milk produced from ewes fed soybean meal exhibited mostly positive score values. These data indicated that the composition of diets had an effect on the physico–chemical parameters of milk, corroborating the findings of Reynolds, Cannon, and Loerch (2006). In addition, it appeared that milk from ewes fed soybean meal was characterised by the highest amount of fat content, while those fed scotch bean meal presented the highest level of lactose (Fig. 1).

Pearson correlation coefficients between the physico–chemical parameters of milk produced from ewes fed soybean meal and scotch bean meal were determined (data not shown). Significant positive correlations ($P \leqslant 0.05$) were observed between fat and protein, which was also reported by Pavic et al. (2002). Similar positive correlations were observed between protein and lactose, protein and ash, and lactose and ash. However, a strong negative correlation was found between the freezing point and the following physico–chemical parameters: ash, lactose, protein, non fat in DM and density.

3.2. Mid infrared measurements

The absorption bands observed in the MIR (4000–900 cm^{-1}) are associated with fundamental valence vibrations of functional groups of the molecule. Most of the spectral information used for the discriminant analysis was located in three wavenumber regions.

The 3000–2800 cm^{-1} spectral region corresponds to C–H bound of methyl and methylene groups of fatty acids. This region was dominated by two strong bands located at 2921 and 2852 cm^{-1} which have been assigned to methylene anti-symmetric and symmetric stretching modes (Casal & Mantsch, 1984), respectively (Fig. 2). Two other weaker bands located at 2956 and 2872 cm^{-1} were assigned to the asymmetric and symmetric stretching modes of the terminal methyl group.

What ever the lactation period, milk samples collected from ewes fed soybean meal presented the highest intensity at 2921 and 2852 cm^{-1} than those collected from ewes fed scotch bean meal. In addition, the spectra showed different shapes as a function of the lactation period. First, it was observed that the $A\gamma_{CH_2}/A\gamma_{CH_3}$ were different between milk produced from ewes fed soy bean meal and those fed scotch bean meal. Furthermore, a slight shift to higher wavelengths was observed for the CH_3 stretching mode of milk samples collected from ewes fed soybean meal.

PCA was applied to the 3000–2800 cm^{-1} and the map defined by the PCs 1 and 5 is shown in Fig. 3. The PC1 which took into account 86.9% of the total variance

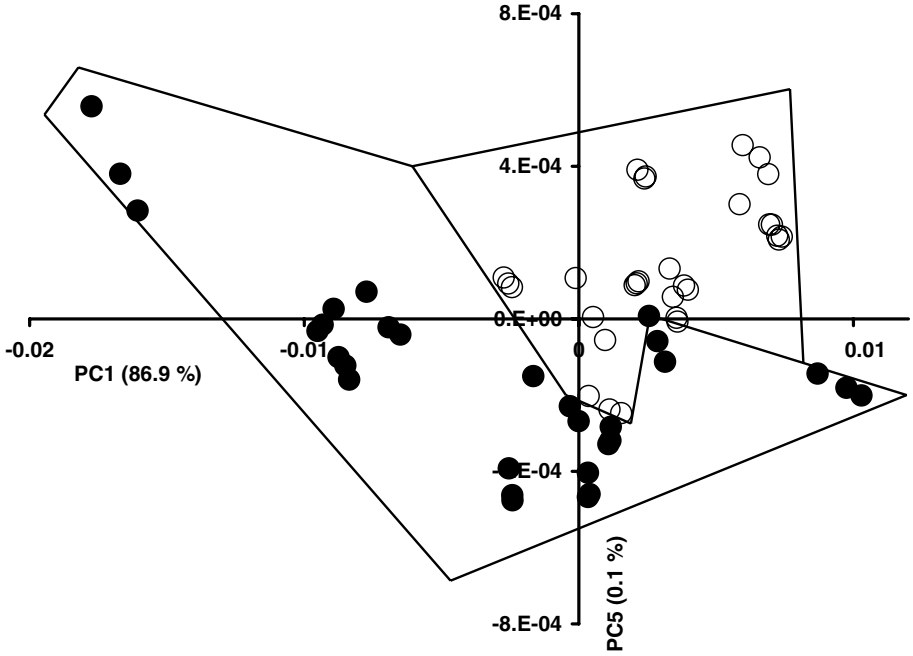

Fig. 3. Principal component analysis similarity map determined by principal components 1 (PC1) and 5 (PC5) of the 3000–2800 cm^{-1} spectral region recorded on Sicilo-Sarde ewe's milk fed soybean meal (○) and scotch bean (●) during lactation period.

allowed a good discrimination between milks produced from ewes fed soybean and scotch bean meals. Indeed, almost all positive score values were observed for ewe's milk fed soybean meal, while those fed scotch bean meal had mostly negative scores.

The spectral patterns corresponding to the PCs provide information about the characteristic absorption bands which explain the discrimination of the investigated milks. These bands could provide valuable structural information about the changes that occurred in the acyl chains during lactation. The spectral pattern 1 (data not shown) showed an opposition between two positives bands at 2919 and 2850 cm^{-1} and three negative peaks at 2956, 2944 and 2881 cm^{-1}. The difference in the $A\gamma_{CH_2}/A\gamma_{CH_3}$ suggested that the viscosity and/or the crystallisation of milk fats changed according to the feeding systems.

The 1700–1500 cm^{-1} was characterised by the presence of bands related to peptides and proteins. Thus, these bands contain some information on the protein and on the interaction of these latter with other components such as ions, water and other proteins. Fig. 4 showed the shape of the spectra obtained after multiple scatter correction (MSC). Contribution to the Amide I band (ν C=O, ν C–N), which was used to investigate the secondary structure of several proteins, can be observed around 1689, 1677, 1656, 1648, 1641 and 1631 cm^{-1}. The absorption bands at 1562, 1548, 1535 and 1515 cm^{-1} are generally assigned to the amide II (δ N–H, ν C–N) vibrations (Fig. 4). The

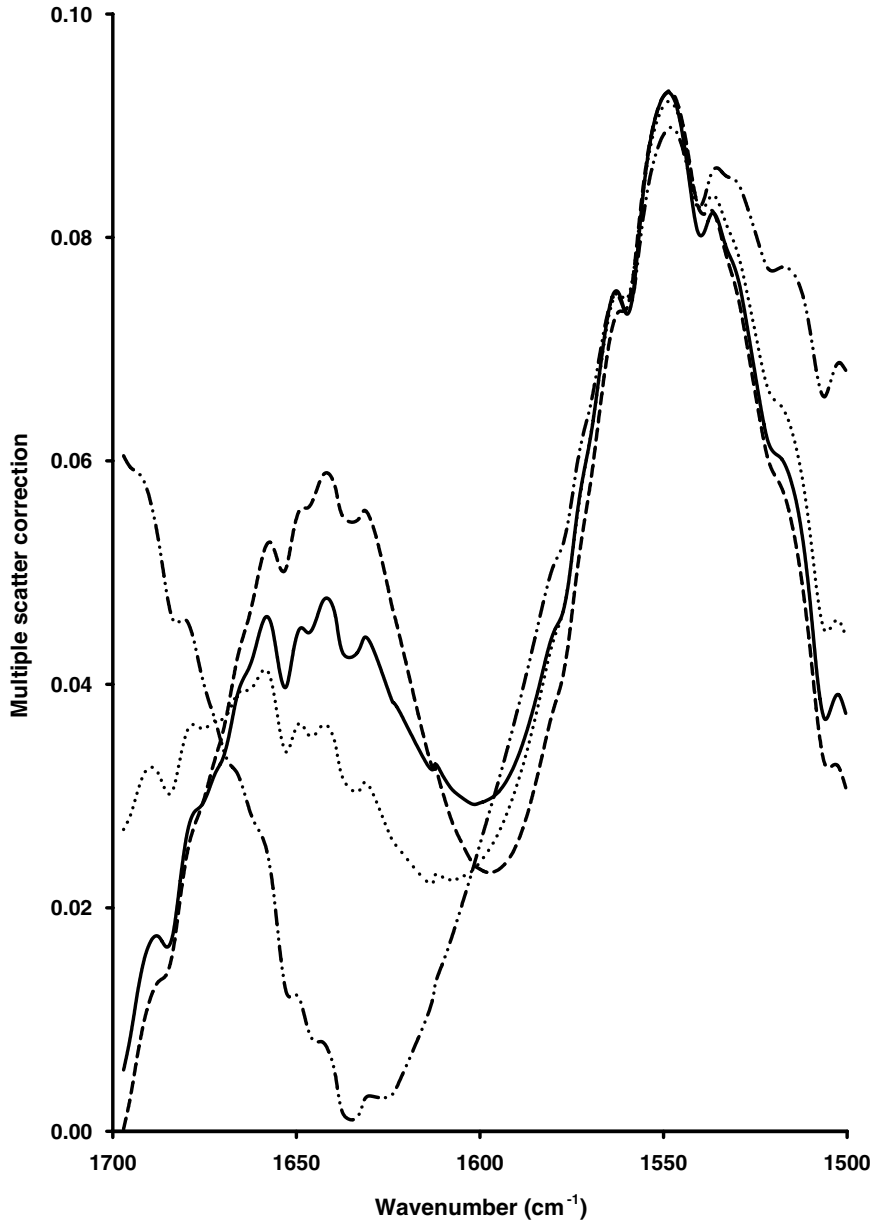

Fig. 4. Multiple scatter correction (MSC) applied to the 1700–1500 cm^{-1} spectral region recorded on Sicilo-Sarde ewe's milk fed scotch bean and soybean meals after 1 (—), (. . .), and 10 (– – –), (—..—..) weeks of lactation, respectively.

absorption band around 1575 cm^{-1} has been attributed to soluble carboxylic acids (Picque, Lefier, Grappin, & Corrieu, 1993).

From Fig. 4, it appeared that the replacement of soybean by scotch bean induced large difference in the amide I region but not in the amide II region of ewes milk produced from scotch and soybean meal throughout the lactation period. We have no explanation of this phenomenon at the moment.

It is worth noting that the interaction of calcium with carboxylate acids presented bands in the 1560–1580 cm^{-1}. Indeed, a decrease in absorption at about 1580 cm^{-1} has already been described by Byler and Farell (1989), studying the interactions of calcium ions with carboxylate groups of proteins. The authors assumed that the calcium ions could bind to the carboxylate groups of the side chains of aspartic and glutamic acids.

PCA was applied to the 1700–1500 cm^{-1} spectral region after MSC (data not shown). According to the PC1 which explain 91.2% of the total variance, milk from ewes fed soybean meal presented mostly positive score values, while those fed scotch bean meal had mostly negative scores. The spectral pattern 1 showed an opposition between 5 positive peaks located at 1575, 1558, 1540, 1521 and 1506 cm^{-1} and 3 negative peaks located at 1658, 1641 and 1631 cm^{-1} (data not shown). The decrease in the intensity of the band observed around 1575 have been attributed to the modification of the nature of anion which interact with aspartic and glutamic acids side chains in caseins (Byler & Farell, 1989).

The region of 1500–900 cm^{-1} called the fingerprint region refers to C–O and C–C stretching modes (1153–900 cm^{-1}). The bands observed at 1461, 1374 and 1236 cm^{-1} (data not shown) are due to bending modes of O–C–H, C–C–H and C–O–H (Paradkar, Sivakesava, & Irudayaraj, 2003; Sivakesava & Irudayaraj, 2001). The bands located at 1041 cm^{-1} (primary alcohol ν C–O) and 1072 cm^{-1} (δ O–H) have been associated to lactose (Iñón et al., 2004; Martín-del-Campo, Picque, Cosío-Ramírez, & Corrieu, 2007). Milk samples from ewes fed scotch bean meal had intense bands at 1041 and 1072 cm^{-1}, and for consequent high level of lactose, than those fed soybean meal. The obtained results confirm those obtained with the physico–chemical analyses as illustrated in Table 1.

Although a slight overlapping was observed between some milk samples collected from ewes fed soybean and scotch bean meals, the similarity map of the PCA applied to the 1500–900 cm^{-1} showed a good discrimination between milk samples (Fig. 5).

The present results were in good agreement with our previous findings (Karoui et al., 2005). Indeed, we have shown that the 1500–900 cm^{-1} spectral region is a fingerprint region allowing a good discrimination of Emmental cheeses produced during winter time and originating from different geographic origin.

4. Conclusion

The present study showed that mid infrared attenuated total reflection spectroscopy can be considered as a promising tool for monitoring changes in ewe's milk according to the composition of diet. However, the technique failed to discriminate milk samples according to the lactation stage. Thus, mid infrared attenuated total reflection spectroscopy appeared to be more sensitive to the feeding systems than to the lactation stage. The best discrimination between milk produced from ewes fed soybean and scotch bean meals was obtained by using the 1500–900 cm^{-1}

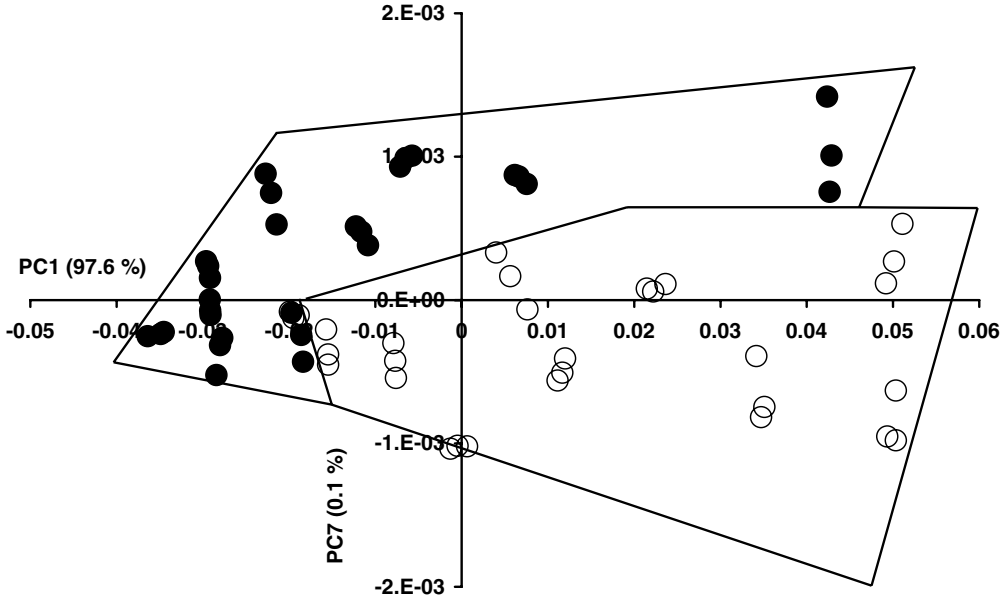

Fig. 5. Principal component analysis similarity map determined by principal components 1 (PC1) and 7 (PC7) of the 1500–900 cm^{-1} spectral region recorded on Sicilo-Sarde ewe's milk fed soybean meal (○) and scotch bean (●) during lactation period.

called fingerprint region. However, further investigations are needed to validate this hypothesis on a large number of ewes and during a longer lactation period. This study will be extended to other ewe breeds and other protein rations which are produced in Tunisia, in order to test the accuracy of this rapid and non-destructive technique for monitoring milk quality according to the feeding systems.

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