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Prediction of fatty acid content in sheep milk by Mid-Infrared spectrometry with a selection of wavelengths by Genetic Algorithms

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ABSTRACT

Sheep breeding is one of the most widespread activities in Sardinia (Italy), and milk produced here is of crucial economic importance for the region. In order to make the milk payment system used in Sardinia more rewarding to the quality of milk, we developed Partial Least Square regression models to predict the concentration of the major fatty acids (measured with a GC-FID reference method) from the Mid-Infrared spectra of hundreds of Sardinian sheep milk samples collected in the period 2011–2013. Genetic Algorithms were used in order to select the most informative spectral subsets and therefore reduce the complexity of the model and in many cases also reduce the prediction error. Models obtained had a good predictive ability, with errors in the range of tenths of a gram of fatty acid on Kg of milk, and an acceptable precision for an immediate introduction on sheep milk payment in Sardinia.

1. Introduction

Sheep milk, in spite of the fact that its production is significantly lower than that of bovine milk (1% against 83% of total milk produced worldwide) (FAOSTAT, 2012), has a relevant importance in the Mediterranean Basin, where climatic conditions and environment are not suitable for cattle raising (Scintu & Piredda, 2007). The island of Sardinia, Italy, where the Sarda sheep breed is reared, is one of the most productive areas, accounting for about 3% of total world sheep milk production (ISTAT, 2012).

The composition of sheep milk differs from that of bovine milk, being richer in nutrients such as proteins and fat (Recio, de la Fuente, Juarez, & Ramos, 2009), and therefore more suitable for cheese production. The economic importance of sheep milk in Sardinia is related to the production of different cheeses, some of them (i.e. Fiore Sardo, Pecorino Sardo and Pecorino Romano) have been awarded the Protected Designation of Origin (PDO) from the European Union (Scintu & Piredda, 2007). Sheep milk price in Sardinia is currently based on milk quality, determined through quality control analysis carried out by the Sardinian Regional Breeders Association (ARA Sardegna). The quality parameters that are taken into account to set milk price are related to security parameters (somatic cell count and bacterial count) and to its composition in fat and proteins. At present, milk payment is mostly based on a grid system that considers different ranges of values for each parameter. Hence, bonus or penalties are applied to the base price of the milk, depending on which range each parameter lies in (Pirisi, Lauret, & Dubeuf, 2007).

Proteins and fat in milk have been found to have different biological activities on the human organism and therefore to have different effects on human health. Proteins (caseins and whey proteins) are in an inactive form in milk but, both during cheesemaking and ripening of cheese and during gastrointestinal digestion of milk and milk products, they release bioactive peptides with biological activities such as antihypertensive, anticytotoxic, antioxidant, antimicrobial, opioid, mineral-binding, and immunomodulatory, depending on their amino acid composition and

Abbreviations: PDO, Protected Designation of Origin; FT-MIR, Fourier-Transform Mid-InfraRed spectroscopy; FA, fatty acids; PLS, Partial Least Squares; GA, Genetic Algorithms; FAME, Fatty Acid Methyl Esters; CLA, Conjugated Linoleic Acid; MUFA, monounsaturated fatty acids; SFA, saturated fatty acids; UFA, unsaturated fatty acids; PUFA, polyunsaturated fatty acids; RMSECV, Root Mean Square Error in Cross-Validation; RMSEP, Root Mean Square Error in Prediction.

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sequence (Recio et al., 2009)). On the other hand, fatty acids (FA) constituting milk triglycerides have different effects on human health. In particular, saturated fatty acids are assumed to be related to the onset of cardiovascular diseases, with lauric, myristic and palmitic acids being the most responsible ones for increasing Low Density Lipoprotein (LDL) cholesterol in the blood (Denke & Grundy, 1992; Mensink, 2013), while monounsaturated fatty acids have beneficial effects by decreasing the risk of cardiovascular diseases (Kris-Etherton, 1999). More specifically, oleic acid (C18:1 *cis*-9), i.e. the most abundant among monounsaturated fatty acids (MUFA), is regarded to as antiatherogenic agent (Molkentin, 2000). Polyunsaturated linoleic and linolenic fatty acids are known to be the precursors of the eicosanoids, substances having strong biological activities in the human body (Calder, 2009; Tilley, Coffman, & Koller, 2001), and of Conjugated Linoleic Acids (CLA). The rumenic acid (i.e. the most abundant CLA isomer) has beneficial roles in humans or animals, such as preventing cardiovascular diseases due to the reduction of LDL cholesterol levels in the blood (Tricon et al., 2004), anti-carcinogenic effects (Ip, Chin, Scimeca, & Pariza, 1991; Palombo, Ganguly, Bistrian, & Menard, 2002) and anti-inflammatory properties (Toomey, Roche, Fitzgerald, & Belton, 2003; Urquhart, Parkin, Rogers, Bosley, & Nicolaou, 2002).

As a consequence of the variety of the positive and negative FA effects on human health, and being the fat/protein ratio in sheep milk higher than that of cow milk (Recio et al., 2009)), a great importance is given in gaining a better knowledge about the acidic composition of sheep milk in relation to its quality. It is thus desirable that sheep milk quality control and payment system could be improved by introducing additional quality parameters related to fatty acids. Setting a fair price in relation to sheep milk quality would encourage breeders to improve the herd management in order to increase the quality of their milk, as it is known that different factors, especially the feeding system, affect milk fat composition (Addis et al., 2005, 2009; Cabiddu et al., 2003).

Pursuing our interest in the assessment and validation of new analytical protocols for the qualitative and quantitative determination of major and minor compounds in sheep milk (Addis et al., 2005; Piga, Urgeghe, Piredda, Scintu, & Sanna, 2009; Piga, Urgeghe, Piredda, Scintu, & Sanna, 2010; Piga et al., 2013) and related dairy products (Addis et al., 2005; Urgeghe et al., 2012), we present the development of fast and cheap analytical methods to determine the individual fatty acid content in sheep milk using Mid-Infrared spectrometry (MIR). MIR is a valid alternative to the traditional gas-chromatographic method, notoriously expensive and time-consuming. The application of Partial Least Squares regression (PLS) (Geladi & Kowalski, 1986) to MIR spectra, up to now performed mainly on bovine milk (Coppa et al., 2014; Soyeurt et al., 2006), allows to relate MIR spectra to fatty acid contents and to build models to be used to determine the composition of future samples.

A selection of informative variables is recommended to build simpler and more robust models and improve the prediction ability (Thomas, 1994). In particular, the use of Genetic Algorithms (GA) (Leardi, Boggia, & Terrile, 1992; Leardi & Lupianez Gonzalez, 1998; Leardi, 2000, 2007, 2009) for selection of informative spectral regions appears to be a useful tool to build a simple, robust and reliable prediction model for the rapid determination of the concentration of each fatty acid or entire categories of fatty acids.

2. Materials and methods

2.1. Sampling

In cooperation with the Sardinian Regional Breeders Association (ARA Sardegna), a total of 250 sheep milk samples was collected from several breeding farms in Northern, Central and Southern areas of Sardinia, covering a period of time ranging from January 2011 to April 2013. In this way it was possible to gather up samples that included variability due to different factors such as geographical areas, season, different herd management and feeding system. In particular, 118 individual sheep milk samples were collected weekly from a sheep breed reared at the experimental farm of AGRIS Sardegna (Northern Sardinia) from January to May 2011, 26 individual samples were collected from the same breed in March and April 2013, 60 bulk sheep milk samples were collected from sheep breeding farms in the central area of Sardinia from February to April 2012, 20 bulk milk samples were collected in the central area of Sardinia in November 2012 and 26 bulk milk samples were collected from as many breeding farms in the Southern area of Sardinia from May to June 2012. All samples were divided into two homogeneous sub-samples and stored at 4 °C. Whereas an aliquot was used to acquire MIR spectrum, the other one was destined to fatty acid analysis and processed to separate milk fat cream within 4 h.

2.2. Instrumentation and reagents

FT-MIR spectra of the sheep milk samples were recorded on a Spectrometer Milkoscan FT6000 (FOSS); the quantitative determination of fatty acid methyl ester profile of sheep milk samples were accomplished on a Gas Chromatograph Varian 3600 equipped with a split/splitless injector, a Flame Ionization Detector (FID), a capillary column SP2560 (100 m × 0.25 mm i.d., 0.20 μm thickness, Supelco Inc.), and controlled by the software Star Chromatography Workstation 6.0 (Varian). The operating chromatographic conditions were: carrier gas: helium; flow: 1 ml min^{-1}; injector split ratio: 1:100; injector temperature: 290 °C; oven temperature program: 4 min at 45 °C, then from 45 °C to 175 °C at a rate of 13 °C min^{-1}, 175 °C isotherm for 27 min, then from 175 °C to 215 °C at a rate of 4 °C min^{-1}, 215 °C isotherm for 35 min; FID temperature: 290 °C. The centrifuge was an ALC mod. 4237 R (ALC International); the thermostated water bath was a Haake D8 (HAAKE, Karlsruhe, Germany).

A standard mixture of both 37 pure fatty acid methyl esters and CLA methyl esters was obtained from Matreya Inc., whereas the methyl esters of C5:0, C9:0, C13:0 and C19:0 fatty acids used as internal standard in the chromatographic quantification were from Sigma Aldrich, Milan, Italy. Potassium hydroxide (for analysis, ACS-ISO) was from Carlo Erba Reagents, Milan, Italy. ACS ISO grade methanol (purity > 99.8%), hexane Laboratory reagent (purity > 95%), sodium bisulfate monohydrate (purity > 99%) were from Sigma Aldrich, Milan, Italy.

2.3. MIR spectra

The MIR spectrum of each sheep milk sample was firstly acquired in duplicate, and then averaged, by using the Spectrometer Milkoscan FT6000 (FOSS). MIR spectra were recorded in the region between 925.92 and 5011.54 cm^{-1}. Since instrumental resolution is 3.858 cm^{-1}, each spectrum consisted of 1060 data points. Fig. 1 shows the overlapped MIR spectra of the 250 sheep milk samples.

The elaboration was performed only on the spectral region between 926 and 3050 cm^{-1} (550 data points), since the region at higher wavenumbers was characterized in part (from 3050 to 3850 cm^{-1}) by a strong instrumental noise and in part (from 3850 to 5012 cm^{-1}) by pure baseline.

2.4. Fatty acids analysis

Milk fat was extracted through the “physical method” proposed by Murphy, McNeill, Connolly, and Gleeson (1990). Milk samples, stored at 4 °C, were heated at 37 °C, gently homogenized and an