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Genetic analysis of the Fourier-transform infrared spectra of bovine milk with emphasis on individual wavelengths related to specific chemical bonds

G. Bittante and A. Cecchinato[1]
Department of Agronomy, Food, Natural Resource, Animals and Environment (DAFNAE), University of Padova, Viale dell'Università 16, 35020 Legnaro (PD), Italy

ABSTRACT

Fourier-transform infrared (FTIR) spectra are used to predict the fat, protein, casein, and lactose contents of milk. These estimates are currently used to predict the individual estimated breeding values of animals. The objective of the present study was to estimate the genetic variation and heritabilities of the milk transmittance spectrum at each individual FTIR wave. Milk was sampled once per cow from a total of 1,064 Italian Brown Swiss cows from 30 herds, sired by 50 artificial insemination sires. The FTIR spectra of all samples were collected within 3 h of sampling from 25 mL of milk. The obtained spectral range comprised wavenumbers 5,000 to 930 × cm^{-1}, corresponding to wavelengths 2.00 to 10.76 μm and frequencies from 149.9 to 27.9 THz, for a total of 1,056 waves. These were acquired using a MilkoScan FT120 FTIR interferometer (Foss Electric A/S, Hillerød, Denmark). Each spectral data point was treated as a single trait and analyzed using an animal model REML method. The results indicated that the transmittance of the bovine milk FTIR spectrum was heritable for most individual waves in the wavenumber interval from 5,000 to 930 × cm^{-1}. Moreover, the transmittance of contiguous FTIR waves was much more highly correlated in terms of the average value and phenotypic variation, compared with genetic variation. In the present study, we characterized 5 regions of the FTIR spectrum that were relevant to the analysis of milk; 2 regions, one in the transition area between the short-wavelength infrared (SWIR) and mid-wavelength infrared (MWIR) divisions of the electromagnetic spectrum (SWIR-MWIR region) and another very short region in the MWIR division (MWIR-2 region), were characterized by very high phenotypic variability in the transmittance of individual milk samples within each wave. This was caused by the absorption peaks of water, which can mask the effects of other important milk components. These regions also showed high genetic variability in transmittance, and the heritability estimates of individual waves were generally very low (with some exceptions). The 3 other identified regions contained many transmittance peaks that represented important chemical bonds; these showed much lower phenotypic and genetic variability in terms of individual waves, but relatively higher and less variable heritability estimates. Among them, the SWIR region (near-infrared) showed a peculiar cyclic pattern of the heritability coefficients of transmittance, the MWIR-1 region was particularly important for the estimation of fat, and the MWIR-LWIR region (also known also as the "fingerprint region") had 3 areas of relatively high heritability. In summary, we found that the transmittance data from the FTIR spectra of milk have genetic variability that may prove useful for the direct genetic improvement of dairy species, rather than only through indirect phenotypic predictions of individual milk quality and technological traits.
Key words: mid-infrared, spectroscopy, heritability, milk

INTRODUCTION

Near-infrared spectroscopy (**NIRS**) and mid-infrared spectroscopy (**MIRS**) have been widely used as secondary methodologies (requiring calibration based on primary wet analyses) for predicting the chemical and technological properties of different materials. The most-promising fields of application include uses in the agricultural products and food industries. For example, Karoui et al. (2010) reviewed the use of MIRS coupled with chemometrics for analyzing intact food systems and exploring the relationships between their molecular structures and quality. According to those authors, the main areas of research to date include quantitatively determining the main components of food and authenticating food products, particularly those produced using traditional technologies in limited production regions. Attempts have also been made to predict the technological and sensorial properties of food, as well as their areas of origin and typicality. However, much more research is warranted in these fields.

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[1]Corresponding author: alessio.cecchinato@unipd.it

The use of the NIRS and MIRS has spread largely because (1) they require little or no sample pretreatment; (2) they are nondestructive; (3) they do not require chemicals or other consumables; (4) the spectra are obtained very rapidly and often automatically; (5) the spectra can be retrieved using portable instruments at the farm, industry, retail, or domestic levels; (6) for some applications (and given specific probes), the spectra can be retrieved from the surface of intact unsampled materials; (7) customized, simplified instruments are available for continuous monitoring "at line" during the processing of food; (8) the data contained within the spectra are very complex and can reflect both physical states and molecular structures; (9) the spectral data are easily stored; (10) given the availability of proper calibrations, one spectrum can be simultaneously used for several chemical/physical predictions; (11) spectral information can provide a very complex description of samples (a "fingerprint") that can be useful in describing the quality and typicality of food; and (12) when a new calibration becomes available for a trait (whether old or new), the stored spectra of samples that are no longer physically available can be reevaluated using this calibration.

One of the most widespread uses of MIRS is to predict the chemical composition and technological properties of milk (Barbano and Lynch, 2006; Woodcock et al., 2008; Brandt et al., 2010). In the cheese-making industry, MIRS is widely used to monitor milk processing and cheese making (Laporte et al., 1998; O'Callaghan et al., 2002; Leitner et al., 2011), to evaluate specific chemical or technological aspects of the products (Payne et al., 1993; Fagan et al., 2007b; Koca et al., 2007; Martín-del-Campo et al., 2007), to predict the sensory traits of cheese (Fagan et al., 2007a; Subramanian et al., 2009), and to assess the individuality and origin of cheese (Pillonel et al., 2003; Karoui et al., 2005a,b).

Fourier-transform infrared (**FTIR**) spectrometry, which is a type of MIRS that allows users to rapidly scan a complete spectrum of electromagnetic waves (Karoui et al., 2010), is commonly used to analyze milk samples that are collected periodically according to the milk-recording schemes of different countries. The International Committee for Animal Recording (ICAR, 2012) has approved FTIR spectrometry as the standardized routine method for analyzing the fat, protein, and lactose contents of milk. Fourier-transform infrared spectrometry has also been proposed for the population-level analysis of other milk components, such as fatty acids (Rutten et al., 2009; De Marchi et al., 2011; Soyeurt et al., 2011), protein fractions (van der Ven et al., 2002; Bonfatti et al., 2011; Rutten et al., 2011), enzymes (Soyeurt et al., 2008b), and minerals (Wu et al., 2009; Soyeurt et al., 2009). Moreover, FTIR has been proposed for the prediction of milk coagulation properties and titratable acidity (Dal Zotto et al., 2008; De Marchi et al., 2009).

Fourier-transform infrared spectrometry-based predictions of milk fat and protein (casein) contents are currently used to estimate the breeding value of animals. However, researchers are also interested in predicting other milk-related selection objectives from the same spectral information, as in the genetic evaluation of FTIR spectrometry-predicted FA (Soyeurt et al., 2007b, 2008a; Rutten et al., 2010), protein fractions (Soyeurt et al., 2007a; Arnould et al., 2009), and coagulation properties (Cecchinato et al., 2009; Bittante et al., 2012). Notably, FTIR spectrometry-based predictions of milk FA are also currently being investigated as indirect measures of an animal's body energy status (McParland et al., 2011) and fertility (Bastin et al., 2012), and NIRS has been proposed as an indirect tool for selecting beef cattle for meat quality (Cecchinato et al., 2011a, 2012).

The measure of some physical property of a biological sample (for example, the absorbance or transmittance of milk at specific wavelengths) is determined by its chemical properties (the presence of specific chemical bonds and the structures of lactose, caseins, whey proteins, and fat, among others). This chemical composition is the result of a physiological process (milk synthesis in the mammary gland) that is subject to genetic control (the major genes and polygenic effects on milk yield). Each step of this process, starting from physical measures of milk and ending with the genetics of the cow, have been extensively studied. Moreover, the same spectral information can be used to predict multiple chemical or technological traits of the animal product, each of which is characterized by different physiological processes and genetic controls. The present study sought to directly link the genetics of a cow and the mid-infrared (**MIR**) transmittance of her milk, and to estimate the genetic variation and heritabilities of the transmittance of individual wavelengths in relation to the area of the spectrum and their phenotypic variability, with a special emphasis on the wavelength characteristics of specific chemical bonds and milk constituents.

MATERIALS AND METHODS

Sample Collection and MIR Spectra Acquisition

A total of 1,200 Brown Swiss cows reared in 30 herds, offspring of 50 AI sires and located in northern Italy, were milk sampled once. After initial data screening and removal of records with incomplete information, 1,064 samples were available for the study. The aver-