PERSPECTIVES OF THE INFRARED AND RAMAN SPECTROSCOPIC TECHNIQUES TO QUANTIFY CONJUGATED LINOLEIC ACIDS (CLA) IN MILK FATS

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Introduction

Recently, the interest in the analysis of conjugated linoleic acids (CLA) has considerably increased because of the numerous potentially positive effects of different CLA isomers on human health. The collective term CLA generally refers to a mixture of conjugated positional and geometric isomers of linoleic (*cis*- 9,*cis*-12-octadecadienoic) acid formed in the rumen by biohydrogenation of polyunsaturated fatty acids. The *cis*-9,*trans*-11-linoleic acid isomer referred to as rumenic acid is generally considered as the major biologically active form among the CLA isomers found in milk fat. Moreover, milk fat from ruminant animals represents the major dietary source of CLA. In this way, the increase of CLA concentration in dairy products has become an important objective in animal nutrition research.

In several countries, farmers and dairy companies are already in the process of commercializing dairy products that are naturally enriched in CLA through the use of specific rations to feed the lactating animals.The development of such activities calls for appropriate analytical methods. The determination of milk fatty acid profile, and in turn of its CLA content, is traditionally made by fat extraction followed by gas chromatography (GC) analysis. This analytical technique gives good results but allows the analysis of only few samples per day. To find a technique that is less tedious and time consuming, it has been decided to test the potential of fast spectrometric techniques. This study concerns a first trial of CLA determination in milk fat by Infrared and Raman spectroscopic techniques.

Aim

The perspectives of FT-Raman, FT-MIR and NIR spectrometry are studied in an effort to develop a rapid method for the determination of the fatty acid profile of Anhydrous Milk Fat (AMF). The AMF samples analysed were obtained from studies focused on the influence of diet on the concentration of CLA in cow 's and ewe's milk. The first step of this study concerns the standardisation of the protocols. Then, several regression algorithms are tested in order to construct the best calibrations.

Procedure

Once the spectra have been obtained by the different spectroscopic techniques, they have been normalised by using the Standard Normal Variate (SNV) method. Calibration models have been constructed using PLS (Partial Least Squares). For that, 55 samples randomly selected are used for model construction (calibration) and 25 have been selected as independent test set for validation (prediction). These two subsets are the same for all the spectroscopic techniques used in this study. The calculations were executed in Unscrambler 9.2™ (CAMO Process AS, Oslo Norway).

Samples

For this study, 34 samples of ewe's AMF and 46 of cow's AMF have been analysed. Gas Chromatography allowed the determination of fat acid profils of the samples. The percentage of CLA (C18,2 conj.) in the samples is inside the 0.34-4.70% range. The samples have been analysed at 40°C.

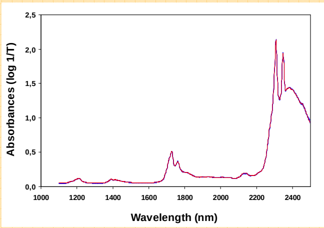
Results

		Calibration	Prediction	PLS factors
RAMAN	R^2	0,95	0,95	3
	RMSE	0,21	0,21	
MIR	R^2	0,96	0,92	7
	RMSE	0,18	0,24	
NIR	R^2	0,95	0,92	7
	RMSE	0,21	0,23	

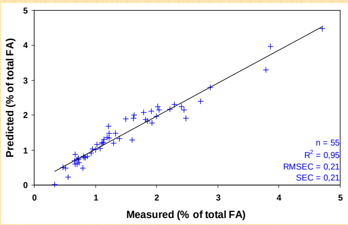
Conclusion

The three spectroscopic techniques studied have shown their capacity to predict the content of CLA for the AMF. Raman and MIR have confirmed the presence of characteristic peaks corresponding to the conjugated double bonds.
In all cases very similar results are obtained, which shows that any technique can be equally used.

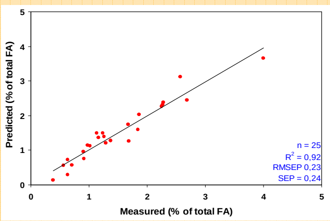
NIR



Example of NIR spectra of cow's AMF (blue) and ewe's (red). This plot shows the similarity between the AMF spectra using NIR.

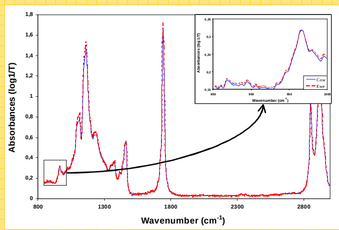


PLS calibration plot for the CLA by NIR spectroscopy.

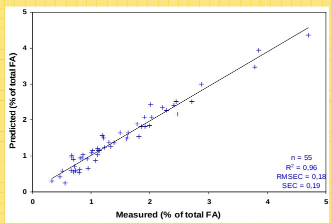


PLS prediction plot for the CLA by NIR spectroscopy.

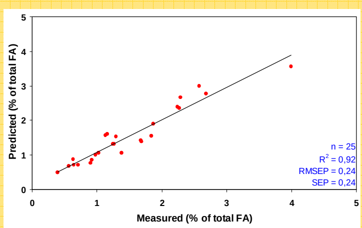
MIR



Example of MIR spectra of cow's AMF (blue) and ewe's (red). The graph at the right part represents the enlargement of the characteristic area for the CLA (850-1000 cm^{-1})

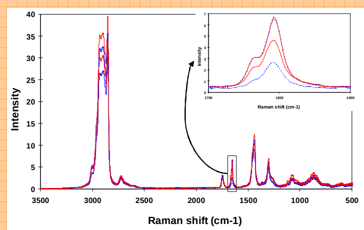


PLS calibration plot for the CLA by MIR spectroscopy.

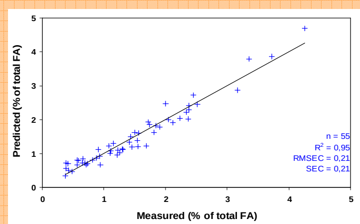


PLS prediction plot for the CLA by MIR spectroscopy.

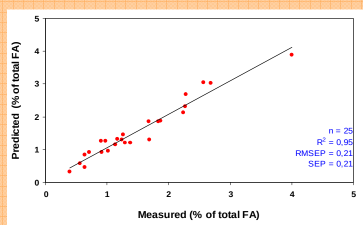
FT-RAMAN



Example of FT-Raman spectra of cow's AMF (blue) and ewe's (red). The graph at the right part represents the enlargement of the characteristic peak for the CLA (1652 cm^{-1})



PLS calibration plot for the CLA by FT-Raman spectroscopy.



PLS prediction plot for the CLA by FT-Raman spectroscopy.

This poster has been presented during the 4th Euro Fed Lipid Congress, which took place the 1-4 October 2006 at the University of Madrid (UCM), Spain.

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