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Analytical Methods

New approach to optimize near-infrared spectra with design of experiments and determination of milk compounds as influence factors for changing milk over time

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ABSTRACT

The optimization of near-infrared spectroscopic parameters was realized via design of experiments. With this new approach objectivity can be integrated into conventional, rather subjective approaches. The investigated factors are layer thickness, number of scans and temperature during measurement. Response variables in the full factorial design consisted of absorption intensity, signal-to-noise ratio and reproducibility of the spectra. Optimized factorial combinations have been found to be 0.5 mm layer thickness, 64 scans and 25 °C ambient temperature for liquid milk measurements.

Qualitative analysis of milk indicated a strong correlation of environmental factors, as well as the feeding of cattle with respect to the change in milk composition. This was illustrated with the aid of near-infrared spectroscopy and the previously optimized parameters by detection of altered fatty acids in milk, especially by the fatty acid content (number of carboxylic functions) and the fatty acid length.

1. Introduction

Milk production plays a predominant economic and social role to the European Union (EU) for several reasons. First, production is often carried out in geographic regions of particular value for their landscape and environment, especially, milk originating from mountaineous areas. Second, dairy farming helps shaping and maintaining land scape and economy in the mountaineous areas. Furthermore, dairy farming is helpful in those mountain areas, which could be at risk of abandonment due to their intrinsic harsh environment. For these reasons, milk production plays an important role going beyond simple cost-benefit analysis. The European Union (EU) has a high interest in protecting product names from misuse and imitation (Tosato, 2013). Currently, the European Union supports two quality strategies based on the valorization of the geographical origins of foods. These strategies are defined by the Regulation (EU) No. 1151/2012, also known as protected designation of origin (PDO) and protected geographical indication (PGI). For milk and cheese production 182 PDO and 36 PGI have been registered so far (Velcovska & Sadilek, 2014).

Food scientists sustain such policy schemes through the development of analytical tools able to enhance the capacity to determine the geographical origin of foods in parallel to its quality. Several analytical approaches have faced this challenge based on (a) mass spectrometry (b) infrared spectroscopy and (c) gas chromatography. Additionally, isotope mass spectrometry (IRMS) is considered one of the most specific analytical tools for exactly this purpose (Scampicchio et al., n.d.).

Near-infrared spectroscopy (NIRS) and mid-infrared spectroscopy (MIRS) have been applied in a broad application field as secondary methodologies (requiring calibration based on primary wet analyses) for predicting chemical and physical parameters of different materials (Huck, 2015). NIR spectroscopy is typically applied for pharmaceutical (Märk, Andre, Karner, & Huck, 2010), agricultural and clinical analysis (Petter et al., 2009). Several reports in literature about quality determination of milk (Downey, 1996; Pillonel et al., 2007; Tsenkova, Atanassova, Itoh, Ozaki, & Toyoda, 2000; Tsenkova, Atanassova, Kawano, & Toyoda, 2001) are available highlighting the benefits of a fast (measurement within a few seconds), non-invasive technique allowing the simultaneous determination of physico-chemical parameters in combination with multivariate analysis (MVA). In general, NIR spectroscopy is concerned with the absorption, emission, reflection and diffuse-reflection of light and is concerned only with overtones and combination modes. NIR is a sister technology to mid infrared and Raman spectroscopy (Druy, Crocombe, & Bannon, 2015). The NIR region may be divided into region I (12,500–8500 cm^{-1}),

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region II (8500–5500 cm^{-1}) and region III (5500–4000 cm^{-1}). The borders of the three regions are not rigorous. Region I has the nick name "the short-wave NIR (SWNIR) region", "near NIR (NNIR) region", or the "Herschel region" (Ozaki, McClure, & Christy, 2006). Due to the high complexity of spectral information caused by overlapping overtones and combination vibrations in most cases in a first attempt spectral pretreatments, and in a second attempt, multivariate analytical (MVA) methods are applied (Martens & Naes, 1989).

Many experiments fail in their purpose because they are not properly thought out and designed in the first place, and in such cases even the best data analysis procedures cannot compensate for the fatal lack of foresight and planning. Failure to design an experiment properly may mean that insufficient results – or at least insufficient results of the right kind – are obtained, or that an unnecessarily large number of measurements are taken. It is even possible to fall into both traps, i.e. to take too many measurements of one kind, and not enough of another. In all such cases the quality of the conclusions drawn will be reduced, and invaluable resources of time, samples, reagents, etc. wasted. (Miller & Miller, 2010)The one-factor-at-a-time (OFAT) approach is often used to characterize the impact of certain experimental parameters on the outcome of an experiment (Daniel, 1973). However, this is suboptimal because the individual runs during an investigation do not cover the design space and hence the degree of information derived from the experiment is low (Czitrom, 1999). Furthermore, interrelationships among different factors can remain concealed resulting in poor models and/or the prediction of false optima (Lipsey, 1990). These disadvantages can be avoided by the use of design of experiments approach in which the runs of an experiment are scattered more evenly throughout the design space (Montgomery, 1984). Design of experiments (DoE) is a revolutionary approach to optimization and screening of experimental parameters. Statistical tools and simple experimental designs for data analysis can provide much information about the investigated system after carrying out only a few experiments. For decision-making in further experiments such information can be the key as well as enabling the development of robust and reliable protocols for chemical synthesis (Santanilla et al., 2015), biological assays (Choi et al., 2013) or analytical methods (Chen, Zhao, Miao, & Wu, 2015). Linking of the DoE approach with modern high-throughput analytical systems, such as HPLC or NIRS, has the potential to maximize the capabilities of these systems and give increased productivity for many applications in research and industry (Maiti et al., 2014), especially in pharmaceutical development (FDA, 2009).

In the present approach it is shown for the first time, how design of experiment based optimized near infrared spectroscopic (NIRS) parameters can be applied to discriminate milk originating from alpine pasture and indoor breeding during October–December 2012. Individual spectral markers will be identified and interpreted accordingly.

2. Materials and methods

2.1. Milk samples

The milk for the optimization of the spectrometric parameters with design of experiments was purchased in the conventional supermarket. 100 g of the Tirol Milk contained 3.5 g fat/100 g, 4.8 g carbohydrates/100 g, 3.3 g proteins/100 g and 0.13 g salt/100 g. For the analysis and further determination of the external influence factors (nutrition, breed, origin etc.) milk samples were collected over a period of three months (October–December 2012). The sampling plan is reported in the Supplementary material Table S1. The milk samples were directly collected from the farmers and transported in special tanks within two days to the laboratory at the University of Innsbruck. Upon arrival, the samples were immediately analyzed or stored at −20 °C until further analyses. The North Tyrolean milk samples were collected and provided by the Agrarmarketing Tirol (AMTirol, Innsbruck, Austria). Raw milk samples from the following North Tyrolean zones were taken under investigation: Innsbruck Stadt, Innsbruck Land, Imst, Kitzbühel, Kufstein, Landeck, Lienz, Reutte and Schwaz (Supplementary Material Fig. S1). Once received, each sample was divided into 50 mL plastic test tubes, frozen at −50 °C and stored until transportation to and further analysis by other project partners. A total of n = 266 milk samples were collected and analyzed (n = 3 design of experiments, n = 84 October, n = 90 November and n = 89 December).

2.2. Near infrared spectroscopy (NIRS)

Near infrared spectra of the milk samples were collected in transflectance mode using a Büchi NIRFlex® N-500 Fourier transform NIR spectrometer and the Fiber Optics Solids cell (Büchi Labortechnik AG, Flawil, Switzerland). This spectrometer is equipped with a tungsten halogen lamp and a temperature controlled extended range Indium-Gallium-Arsenide (InGaAs) detector at 30 °C. Samples were equilibrated for 2 h to room temperature in different water baths to unfreeze slowly. A fiber optic probe of 2 m length was used with two embedded fiber-bundles, one with 2.0 mm diameter for the light beam and one with 3.5 mm diameter for the light collector. The NIR spectra were collected from 10,000 to 4000 cm^{-1} (1000–2500 nm) at a spectral resolution of 8 cm^{-1}. The absolute wavelength accuracy was ±2 cm^{-1} and the relative reproducibility 2.0 cm^{-1}. Each sample was analyzed three times with 64 scans per spectra. The three received spectra were averaged to a single spectrum. For recording the spectra the related NIRWare® (Version 1.4.3010) software package (Büchi Labortechnik AG, Flawil, Switzerland) was used. The background was recorded with 64 scans using a Spectralon® reflector (Labsphere, Munich, Germany).

2.3. Statistics

The design of experiments optimization concerning the full factorial design was carried out using Design-Expert® Ver. 9.0.6.2 (Stat-Ease©, Inc. Minneapolis, MN, USA). All the other chemometric analyses including spectral data pretreatment and principal component analyses (PCA) were performed with Unscrambler® X Ver. 10.3 (Camo Software, Oslo, Norway).

2.3.1. Experimental design

For the identification of the optimal parameters a three-level full factorial design was applied to near-infrared specific parameters. Based on literature research and discussion with near-infrared specialists factors and response variables for the optimization have been determined. These factors are (1) the layer thickness of the transflexion adapter, (2) the number of scans and (3) the ambient temperature of the samples during the measurement (Table 1). The experimental design is balanced, i.e. each factor level exists to the same extent, and thus guarantees the orthogonality of the design. Milk samples have been kept at constant temperature (±0.5 °C) during experimental period using a water bath Grant GD120 (Grant Instruments Ltd, Shepreth, UK). Response variables are so called quality parameters of spectra and included the absorption intensity, signal-to-noise-ratio and the reproducibility of the spectra. Using a bipolar scale with 11 steps response variables were assessed for each spectra. The range of the scala is from −5 to +5 (Table 1). Lack-of-Fit tests were performed resulting