



Infrared spectroscopy: A potential tool for analysis of milk and milk products

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Abstract

Infrared spectroscopy involves the interaction of infrared radiation with matter. It covers a range of techniques, mostly based on absorption spectroscopy. As with all spectroscopic techniques, it can be used to identify and study chemical substances. Samples may be solid, liquid, or gas. The method or technique of infrared spectroscopy is conducted with an instrument called an infrared spectrometer (or spectrophotometer) to produce an infrared spectrum. In dairy industry, IR is useful tool for analysis of milk and milk products. It is mainly used for compositional analysis, adulteration detection, quality monitoring of the milk as well as for milk products.

Keywords: infrared, milk products, adulteration

Introduction

There is an increasing demand of the consumers and actors of the food industry sector to have means of measurement allowing the characterization of milk and dairy products. Authenticity of these products is an important issue for milk processors, retailers, regulatory authorities and consumers. It is also valuable for ensuring fair competition and as a mean of protecting consumers against fraud due to mislabeling. Conventional chemical methods are not able to determine the regional provenance of dairy products unambiguously. Therefore, alternative techniques such as spectroscopic have potential advantages as tools for evaluation of identity of such products. The term 'spectroscopy' encompasses a range of techniques for acquiring information on atomic and molecular structure. These techniques are based on the interaction of electromagnetic radiation (light) with atoms or molecules, where this interaction involves absorption, emission, reflection or scattering of incident electromagnetic radiation (Nawrocka and Lamorska, 2013) [49].

Infrared spectroscopy

Infrared radiation was discovered by F.W Herschel in 1800. This is an electromagnetic radiation extending from 780 nm to 1 mm. The infrared radiation is the region of the electromagnetic spectrum between the visible (VIS) and microwave wavelengths. The infrared region of the electromagnetic spectrum spans from 14,000 to 50 cm^{-1} . The infrared region is divided into three segments *viz.* near infrared (NIR), mid infrared (MIR), and far infrared (FIR). This division is on the basis of types of vibration that occur in each region. However, NIR and MIR are most widely used in the spectroscopy (McLaughlin and Glennon, 2011) [47]. Infrared spectroscopy involves the interaction of infrared radiation with matter. It covers a range of techniques. As with all spectroscopic techniques, it can be used to identify and study chemicals. Samples may be solid, liquid, or gas. An IR spectrum can be visualized in a graph of infrared light absorbance (or transmittance) on the vertical axis vs. frequency or wavelength on the horizontal axis.

Typical units of frequency used in IR spectra are reciprocal centimeters (wave numbers), with the symbol cm^{-1} . Units of IR wavelength are commonly given in micrometers (formerly called "microns"), symbol μm , which are related to wave numbers in a reciprocal way. The method or technique of infrared spectroscopy is conducted with an instrument called an infrared spectrometer (or spectrophotometer) to produce an infrared spectrum (McLaughlin and Glennon, 2011) [47].

Near infrared spectroscopy

The nominal range of wavelengths for NIR is between 780 and 2500 nm (14000 to 4000 cm^{-1}). NIR induces transitions in the vibrational energies of the molecules and used for quantitative analysis (Delgadoillo *et al.*, 2012) [19].

Mid infrared spectroscopy

The nominal range of wavelengths for MIR is between 2500 and 25000 nm (4000 to 400 cm^{-1}). The MIR induces fundamental vibrational transitions in the molecules and mainly used for qualitative analysis.

The MIR region is a very interesting region of the spectrum for the study of organic compounds, because the absorption bands are due to the specific vibration of a particular bond in functional group.

(Karoui *et al.*, 2010; Zhang *et al.*, 2011) [35, 70].

Instrumentation

Nearly all modern MIR spectrometers are Fourier transform (FT) based instruments with different interferometer designs. In contrast, monochromator/detector principles in scanning NIR spectroscopy are variable. Besides different spectrometer types with moving parts, like grating instruments and FT spectrometers with Michelson or polarisation interferometers, fast-scanning instruments without moving parts such as diode-array, acousto-optic tunable filters (AOTF), and light-emitting diode (LED) systems are available (Lin *et al.*, 2009; Siesler, 2008) [12, 61].

Basic principle

Infrared spectroscopy is amenable to quantitative analysis applications, because the intensities of the bands in the spectrum are proportional to the concentration of their respective functional groups, as Lambert-Beer law shows ($A \propto b \cdot c$), where A is the absorbance of the band, b is the path length cell, c is a molar proportionality constant called molar absorptivity which is characteristic of each functional group and c is the concentration of the functional group. Absorption of energy by a molecule can cause the molecule to translate or rotate or it can cause specific groups within the molecule to vibrate or some electrons of the molecule to get excited (Karoui *et al.*, 2010)^[35].

IR spectroscopy monitors the vibrations exhibited by molecules under IR radiation. In stretching vibrations, the atoms move in the same direction (symmetric) or opposite directions (asymmetric). Bending vibrations include scissoring, rocking, twisting and wagging (Nawrocka and Lamorska, 2013)^[49]. The chemical bonds vibrate at specific frequencies, which are determined by mass of constituent atoms, shape of the molecule, stiffness of the bonds and periods of associated vibrational coupling (Khoshhesab, 2012)^[38].

Fourier Transform Infrared (Ft-Ir) Spectroscopy

Dispersive mid-infrared spectroscopy has scarcely been applied to food studies, as a result of the difficulties in sample preparation and handling and because the dispersive instruments are not equipped with the appropriate data acquisition and processing systems to provide quantitative information in a readily usable form. However, since the early 1970s, FTIR spectroscopy has replaced the dispersive instruments and has been widely extended to record infrared spectra. With the FTIR technique, the sample is irradiated with a continuous broad band of radiation covering the spectral region to be examined, rather than the individual wavelengths generated by grating and/or prism systems used in conventional infrared spectroscopy (Cozzolino, 2012)^[17].

The FTIR spectrometer is based on the Michelson interferometer theory. The FTIR is consisting of IR source, laser, beam splitter, fixed mirror, moving mirror, laser detector and IR detector. FTIR also has a number of other advantages, i.e., improved signal to noise ratio, multiplexing capabilities, a significant reduction in scan times, higher energy throughput and superior wavelength accuracy than dispersive-based instrumentation (Subramanian *et al.*, 2011)^[64].

Attenuated Total Reflectance (Atr) Spectroscopy

In conventional IR spectroscopy sample-handling complications arises oils and fats. To overcome this problem, Attenuated total reflectance (ATR) IR spectroscopy was developed in the early 1960s and formerly known as internal reflection spectroscopy (IRS). This spectroscopy utilizes total internal reflection phenomenon. The IR beam travels inside the crystal and a standing wave of radiation, called the evanescent wave, is created. The evanescent wave is absorbed by the sample and is passed back due to total internal reflection. The IR light then is detected by the detector and converted into infrared spectra (Khoshhesab, 2012)^[38].

The ATR crystals are made from materials that have a very high refractive index and low solubility in water. The commonly used material for the ATR crystal are zinc selenide (ZnSe), silicon and diamond. They have various spectral ranges and different

refractive index and can be selected for different samples. Two configurations of ATR accessory are available. In a single bounce-ATR, a single internal reflection occurs using a prism whereas Multi-Bounce ATR, undergoes multiple internal reflections (up to 25) using special prisms (Karoui *et al.*, 2010)^[35].

Chemometric

The term “chemometrics” is derived from two ancient Greek words *chemeia* (meaning chemistry) and *metros* (meaning measure) (Kiralj and Ferreira, 2006; Bansal *et al.*, 2014)^[39, 6]. Chemometrics can be defined as the science of extracting chemically relevant information from multivariate data by using statistical techniques to reduce the dimensionality of the dataset. In these different types of tools are used to calculate the data like PLS (Partial Least Square), PCR (Principal components regression) combined with different softwares (Capuano and van Ruth, 2012)^[11].

Applications in Analysis of Milk and Milk Products

It is well known that MIR and NIR spectroscopy can be applied as alternative method in quantitative measurement of different food constituents, sensory parameters of food products, natural products analyses, determination the geographical origin of the food products, bacterial detection, adulteration detection and authentication studies (Javidnia *et al.*, 2013; Marchi *et al.*, 2014)^[33, 43].

Milk

Compositional analysis

Grewal *et al.* (2017)^[28] applied FTIR analysis for physicochemical changes (conformational rearrangements of proteins, protein-protein and protein-lipid interactions) in UHT milk during accelerated storage. Roza-Delgado *et al.* (2017)^[58] used matching portable hand-held MicroPhazir™ (MP) NIRS (1600-2400 nm) instruments for in situ monitoring 542 fresh milk samples for its composition that provide indicators of milk composition at farm level. Fleming *et al.* (2017)^[25] were detected milk fatty acid content with mid-infrared spectroscopy in Canadian dairy cattle using differently distributed model development sets. Gao *et al.* (2017)^[27] used vibration spectroscopic technique for species identification based on lipid characteristics. Wang *et al.* (2016)^[67] studied the genetic and environmental variation in bovine milk infrared spectra (925 to 5,008 cm^{-1}) and found that diacylglycerol O-acyltransferase, stearoyl-CoA desaturase, κ -casein and β -lactoglobulin polymorphisms were known to have a large effect on milk composition. McDermott *et al.* (2016)^[46] were evaluated the effectiveness of MIR (900 to 5,000 cm^{-1}) in predicting milk protein and free amino acid (Lys, Val, Glu, Gly, Asp, Arg, and Ser) composition in bovine milk. Baum *et al.* (2016)^[9] carried rapid quantification of casein in skim milk using FTIR. Stocco *et al.* (2016)^[63] were assessed the variability in the major mineral (Ca, P, K, and Mg) components of buffalo milk and also estimated the effect of certain environmental sources of variation on the major minerals during lactation by using FT-IR spectroscopy. FT-MIR becomes a powerful technology to quantify milk components and/or to permit a screening of the dairy cattle population based on different milk characteristics interesting for different purposes (Soyeurt, 2010)^[62].

Detection of adulteration

Jaiswal *et al.* (2015) [32] reported use of ATR-FTIR in combination with chemometrics for detection and quantification of soymilk adulteration in milk on the basis of protein characterization. FT-MIR coupled with chemometric methods has been applied for determination of sucrose in raw milk. (Bassbasi *et al.*, 2014) [7]. MIR Spectroscopy was evaluated by Santos *et al.* (2013) [59] as a rapid method for detection and quantification of milk adulterated with whey, hydrogen peroxide, synthetic urine, urea and synthetic milk and spectra were recognized by Soft Independent Modeling of Class Analogy (SIMCA) and PLSR. FT-IR was developed to determine the concentration of sodium bicarbonate, sodium citrate and cheese whey in raw milk (Cassoli *et al.*, 2011) [12]. Parat *et al.* (2017) [55] validated of MIR for macronutrient analysis of human milk. Nicolaou *et al.* (2010) [50] used FTIR spectroscopy and multivariate analysis for the detection and quantification of different milk species (cow, goat and sheep) as well as for the quantitative assessment of the adulteration of sheep, goat, and cow milks. FTIR spectroscopy (1745 cm^{-1}) has been applied to differentiate between goat and sheep milk (Pappas *et al.*, 2008) [54].

Monitoring of quality

McDermott *et al.*, (2016) [46] studied the effectiveness of MIR spectroscopy to predict the color of bovine milk (yellowness index; b^*) and the relationship between milk color and traditional milk quality traits. Shuso *et al.* (2007) [60] constructed a NIR (600–1050 nm) spectroscopic sensing system for online monitoring of milk quality during milking.

Butter

Compositional analysis

Zhao *et al.* (2015) [71] used vibrational spectroscopy and multivariate data analysis for prediction of naturally-occurring, industrially-induced total *Trans* fatty acids in butter.

Detection of adulteration

Terouzi *et al.* (2017) [65] used ATR-FT-MIR region of $4000\text{--}600\text{ cm}^{-1}$, combined with multivariate analysis to quantify the mashed potatoes (0–35%) content in a binary mixture with traditional cow's butter. Cuibus *et al.* (2015) [18] applied ATR-FTIR for the discrimination of butter samples adulterated with solid fraction of palm oil. Nurrulhidayah *et al.* (2015) [51] used FTIR-ATR spectroscopy based metabolite fingerprinting as a direct determination of butter adulterated with lard. Ucuncuoglu *et al.* (2013) [66] determined the margarine adulteration in butter used for bakery products, by Raman and near-infrared (NIR) spectroscopies.

Nurrulhidayah *et al.* (2013) [52] monitored the detection and quantification of butter adulteration with chicken fat using FT-IR spectroscopy, combined with chemometric of PLS at the frequency regions of $1200\text{--}1000\text{ cm}^{-1}$.

Koca *et al.* (2010) applied temperature-controlled ATR-MIR spectroscopy for rapid estimation of butter adulteration with margarine fat at levels ranging from (0–100%) (v/v) and found that the information-rich spectral regions were at $3050\text{--}2800$ and $1400\text{--}850\text{ cm}^{-1}$. Heussen *et al.* (2007) [30] explored use of NIR spectroscopy and multivariate modelling to obtain a rapid method.

That can detect adulteration of butter fats with cheaper vegetable fats.

Ghee

Characterization of spectra

Pandey and Jariwala (2015) [53] reported preliminary work on IR spectroscopy for *trans* fatty acids in ghee and possibility to detect adulteration in ghee. Application of FT-NIR and FT-MIR spectroscopy in reflectance mode and transmittance mode for spectral study of ghee was evaluated by Antony *et al.*, (2016) [2]. They also reported FT-NIR and FT-MIR spectra of ghee (Antony *et al.*, 2017a; 2017b) [3, 4].

Cheese

Compositional analysis

Filho and Volery, (2005) [24] were used NIR to quantify total solid contents of fresh cheeses. Mid-infrared (MIR) spectroscopy was used in transmission mode for determining some chemical parameters (Fat, total nitrogen, water soluble nitrogen, non-protein nitrogen, sodium chloride and pH) of European Emmental cheeses (Karoui *et al.*, 2005) [36]. Blaquez *et al.* (2004) used NIR reflectance spectroscopy to predict moisture, fat and inorganic salts in processed cheeses.

Adulteration

Recent developments in FTIR spectroscopy came out with a study to authenticate Romanian cheeses using ATR-FTIR along with PLSR. The results proved that FTIR-PLSR model is a reliable, non-destructive technique for a rapid quantification of palm oil adulteration in cheese (Cuibus *et al.*, 2014).

Coagulation properties

Cipolat-Gotet *et al.* (2012) [16] compared mechanical and near-infrared methods for assessing coagulation properties of 913 Brown Swiss cow's milk to be evaluated during the cheese-making.

Sensory and textural characteristics

Kraggerud *et al.* (2013) [41] predicted of sensory quality of cheese during ripening from chemical and spectroscopy measurements. Downey *et al.* (2005) [22] evaluated the potential of NIR to predict maturity and sensory attributes of 24 Cheddar cheeses produced using five renneting enzymes and stored at 4°C for up to 9 months.

The authors showed that NIR spectroscopy has demonstrated several sensory attributes namely crumbly, rubbery, chewy, mouth coating and mass forming with sufficient accuracy to be industrially useful.

Ripening condition

Subramanian *et al.* (2011) [64] used FT-IR ($4000\text{--}700\text{ cm}^{-1}$) spectroscopy to monitor amino acids, organic acids, and changes during ripening. NIR could be a suitable technique for the evaluation of the shelf-life in which Crescenza freshness is maintained (Cattaneo *et al.*, 2005) [13].

Cheese yield

Ferragina *et al.* (2013) [23] used FT-IR spectroscopy to predict cheese yield and nutrient recovery or whey loss traits from unprocessed bovine milk samples.

Milk powder

Compositional analysis

Holroyd *et al.* (2013)^[31] used in- and on-line near infrared spectroscopy for compositional measurement of milk powder. Maria *et al.* (2011)^[44] classified and determined the total protein in 38 samples of milk powder using NIR spectrometry (714–2500 nm) and SIMCA. FT-IR spectroscopy was used to examine the conformation of proteins in spray-dried milk protein concentrate (Kher *et al.*, 2007)^[37]. Wu *et al.* (2008)^[69] used Short-Wave NIR Spectroscopy (800- to 1,025-nm region) of 350 samples milk powder for brand identification and component analysis. Wu *et al.* (2007)^[68] used IR Spectroscopy technique for the nondestructive measurement of fat content in milk powder. Nagarajan *et al.* (2006)^[48] detected direct moisture content (4–10%) in 45 samples (prepared in the laboratory) of milk powder using near infrared spectroscopy (800–2500 nm).

Adulteration detection

Fu *et al.* (2014)^[26] detected melamine (peak of 1473.8 nm) in milk powders based on NIR (990–1700 nm) hyperspectral imaging and spectral similarity analyses. Roman and Sergey, (2011) detected melamine by using mid- and near-infrared (MIR/NIR) spectroscopy in dairy products including liquid milk, infant formula, and milk powder. Mauer *et al.* (2009)^[45] used the PLSR methods along with FTIR to correlate the infrared spectral data to melamine concentration and concluded that the NIR and the MIR method enable rapid detection of 1 mg/kg melamine in infant formula powder.

Fermented products

Armelle *et al.* (2011)^[5] predicted acidity parameters (pH and titratable acidity) simultaneously in Kefir using near IR spectroscopy. MIR Spectroscopy can also used for identification of lactic acid bacteria in cheese by using 4000-700 cm⁻¹ wave number range (Karoui *et al.*, 2010)^[35]. Robertson. (2014)^[56] used NIR rapid testing for detecting melamine adulteration in yogurt candy.

Other application

Applications of infrared spectral analyses have also increased in other food product quality analysis, and it has been widely used to predict the attributes of grains (Jing *et al.*, 2010)^[34], tea (Chen *et al.*, 2006)^[15], honey (Chen *et al.*, 2011)^[14], fruits and vegetables (Bauer *et al.*, 2008)^[8], beverages (He *et al.*, 2007)^[29], oils (Downey *et al.*, 2003)^[21], eggs (Abdel-Nour *et al.*, 2011)^[1], meat (De Marchi, 2013), and other agricultural products, in order to substitute other commonly used destructive methods.

Advantages and limitations

Advantages and limitations of IR spectroscopy are summarized by (Subramanian *et al.*, 2011)^[64].

Advantages

- Simplicity, sensitivity and speed of detection
- High throughput (ability to analyze several samples in a short time)
- Possibility of non-destructive analysis depending on the application
- Requirement of relatively low sample volumes.
- Possibility of simultaneous analysis of multiple analytes.

- Less use of hazardous solvents that pose environmental and health hazards
- Relatively low operational cost.
- Capability of on-site and in-line analysis.

Limitations

- Cannot detect atoms, monatomic ions, elements, inert gases and diatomic molecules.
- Calibration analysis is indirect and requires reference method and constant updating.
- All dairy products are complex mixtures, therefore IR spectra are complicated.
- Most dairy products contain a high level of moisture (except powders), which has a strong absorption band that can mask certain important signals.
- Changes in environment can occur between samples, causing uncertainties in the spectra.

Conclusion

The application of IR spectroscopy in the dairy sector looks back on a long history. Rapid analyses without the need of sample preparation presents one of the main advantages of this technique. Therefore, this technique meets the requirements of industrial applications for continuous quality control and process monitoring.

IR spectroscopy is capable of rapidly determining composition and characteristics of dairy products and can be a powerful tool for both industrial and research applications. With numerous developments in FT-IR spectroscopy and several applications still unexplored, the future of IR spectroscopy in analysis and quality assurance is promising.

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