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NEAR INFRARED SPECTROSCOPY (NIRS) COUPLED WITH CHEMOMETRIC TOOLS USED FOR FOOD PRODUCTS ADULTERATION DETECTION

Lucija Longin, Franjo Grbeš, Maja Benković, Tamara Jurina, Davor Valinger, Ana Jurinjak Tušek*, Jasenka Gajdoš Kljusurić

University of Zagreb, Faculty of Food Technology and Biotechnology, Pierottijeva 6, 10000 Zagreb, Croatia

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Summary

The growing problem of food products adulteration requires a rapid and simple method for adulteration detection. Adulterants, which can range from plant material to natural and synthetic colours and compounds have a severe influence on customer trust as well as public health risks. Food safety regulations are being tightened and expanded, requiring a more thorough examination of hazards and risk management solutions. Due to its advantages, near infrared spectroscopy has been recognised as an effective method for food adulteration detection. In this work, an overview of the basics of NIR spectroscopy, NIR data analysis and the use of NIR for food adulteration detection are discussed.

Keywords: NIR spectroscopy, food adulteration detection, chemometrics

Introduction

Food is one of the basic human needs. This fact offers many opportunities in the field of food production, but, at the same, can lead to possible threats in the form of fraud, i.e. food adulteration. High-quality foods such as honey or extra virgin olive oil are increasingly becoming targets of counterfeiting. In practice, high-value foods are usually compromised by cheaper, commercially available foods or product ingredients of similar composition (Drabova et al., 2019). Adulterated foods are often labelled as natural and are priced the same as pure foods, which is wrong and unfair to consumers. Although the addition of cheaper components does not pose a health risk, such practices affect market growth and undermine consumer confidence (Downey, 2013). Due to the growing consumer and market demand for better food quality, there is a need for quick, easy and accurate analytical methods to assess the quality and authenticity of food products. Near infrared spectroscopy (NIRs) proved to be one of such method (Bázár et al. 2016). The advantage of NIR spectroscopy as a non-destructive method is that very little or no prior sample preparation is required, no reagents are needed and therefore no waste is generated. Due to the large amount of data obtained and various overlaps in the data, the results must be processed using methods of multivariate analysis. NIR spectroscopy is sensitive to changes in the chemical and physical properties of the sample under investigation and can therefore be used to verify the authenticity and origin of various products. In order to detect and prevent food manipulation and to question the authenticity of individual food products in a timely manner, several studies conclude that in the future it would be desirable to promote the development of rapid, simple and non-destructive methods and emphasize that the application of NIRs should be further explored to develop specific models for detection of adulteration. In this paper, some basic information about NIR spectroscopy, NIR data analysis and the application of NIR for food adulteration detection and analysis are presented.

NIR spectroscopy

Near-infrared spectroscopy is based on the absorption of electromagnetic radiation with a wavelength in the range of 780 - 2500 nm (Figure 1), where the absorption bands correspond mainly to the overtones and vibrational combinations of molecules. The infrared region was discovered in 1800 by F. W. Herschel when he projected light through a prism and measured the relative thermal effect of individual parts of the spectrum. In this way, he discovered that the maximum thermal effect is far outside the visible range.

The intensity of the NIR absorption band depends on the change in dipole moment that occurs during a vibration. The hydrogen atom is the lightest and therefore shows the strongest vibrations and the largest displacement of bonds (C-H, N-H, O-H, S-H).

*Corresponding author: ana.tusek.jurinjak@pbf.unizg.hr

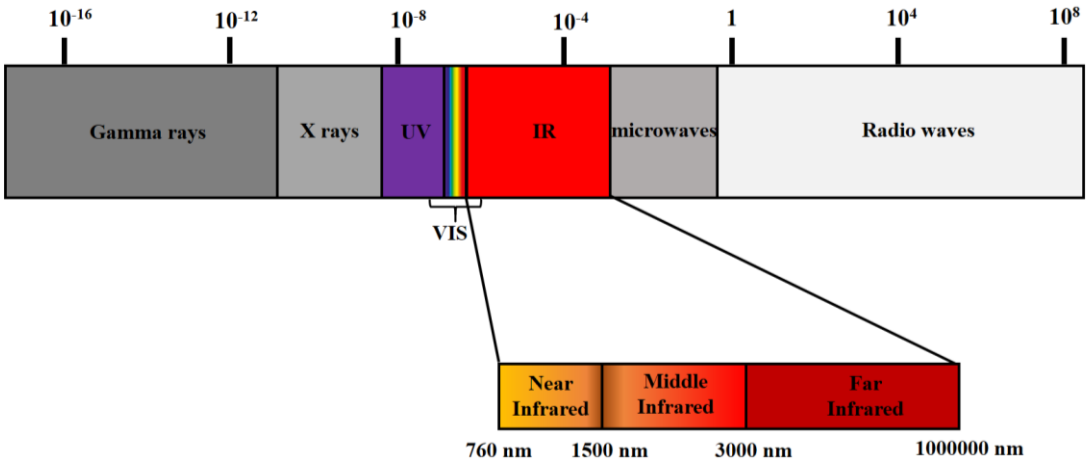

Fig. 1. Electromagnetic spectrum (according to AZO Optics, 2019)

Like any radiation, NIR behaves like a wave with the property of simple harmonic motion, which can be defined by two properties: vibrational frequency and wavelength (Ozaki et al., 2018).

The chemical bonds between atoms in molecules oscillate and to a first approximation, these oscillations behave like simple harmonic motion. The motion of each atom can be treated as an independent oscillation with respect to a fixed centre of mass of the molecule, rather than the mass of the corresponding source. The frequency of oscillation is a function of the mass of two atoms m_1 and m_2 and the bond strength k, and there is a parabolic relationship between potential energy and interatomic distance. Interactions between atoms in different molecules change the vibrational energy states, causing absorption bands to shift and new ones to appear due to differences in the crystal structure. This allows the detection of crystalline forms and the determination of the physical properties of the substance (e.g. density, viscosity and particle size in suspension) (Blanco and Villar, 2001). NIR spectroscopy is a unique tool for studying hydrogen bonding, inter- and intramolecular interactions and hydration, and also provides information on transition of electrons form one *d* orbital to another (*d*-*d* transition) and charge-transfer (CT) transitions. NIR spectroscopy is suitable for non-destructive and *in situ* analysis (Ozaki et al., 2018) and can be useful when it is necessary to acquire data in real time with a reduced possibility of contamination or destruction of the sample (Findlay and Bugay, 1998). NIR is used in monitoring the quality of fruit juices, butter, flour, bread, sugar, edible oils, fish, meat, i.e. in almost all technologies of the food industry. It is possible to perform non-destructive analysis using IR spectroscopy, but if attenuated total reflection or photoacoustic spectroscopy is used, there is no other choice than NIR spectroscopy if it is to be measured in a non-destructive way. NIR spectroscopy allows contactless analysis and analysis with optical fibre probes, which is why it can be used in hazardous environments and the probe can be manipulated remotely. This is one of the reasons why NIR spectroscopy is suitable for online analysis (Ozaki et al., 2018). NIR spectroscopy has been successfully used for qualitative and quantitative analysis. NIR spectra are characterized by a large signal to noise ratio, and the position in the intensity of the bands can be determined very accurately, which is very important for quantitative analysis. The bands in NIR spectra are less informative and may overlap in many cases. Therefore, the obtained data must be processed using multivariate analysis methods (Jednačak and Novak, 2013).

NIR spectra analysis

The analysis of NIR spectra is performed using chemometric methods. Chemometrics is defined as a chemical discipline that uses mathematical and statistical methods to design or select the optimal metrological procedure or experiment and to obtain maximum information by analyzing the obtained data. The results of spectroscopic measurements in complex systems such as food matrix are often ambiguous. Qualitative and quantitative analyses in such complex systems can be problematic, time-consuming, and complicated because of overlapping spectral

responses. The solutions before the implementation of chemometrics were the previous separation of components or increasing the resolution of the spectrum. With the implementation of chemometric techniques, the path of quantitative and qualitative analysis became easier and faster. Spectroscopic techniques in combination with chemometric methods are used for process monitoring and quality control management (Matijević and Blažić, 2008). The statistical methods often used in the analysis of the spectral data are: Principal Component Analysis (PCA), Canonical Correlation Analysis (CCA), Factorial Discriminant Analysis (FDA), Principal Component Regression (PCR), Common Components and Specific Weights Analysis (CCSWA), Partial Least Squares (PLS), and Artificial Neural Networks Method (ANNs) (Karoui et al., 2003).

Principal component analysis is probably the most widely used multivariate statistical method and is considered to be the technique that has changed the way data analysis is viewed (Brereton, 2007). Principal component analysis allows for qualitative analysis and grouping of data without a fixed physical model. It is also used to quickly assess data structure prior to detailed analysis or quantification of a physical or chemical process (Jednačak and Novak, 2013). PCA processes raw data representing samples described by several dependent variables, which are, in most cases, correlated with each other. This method is based on determining the correlations between individual variables by grouping the samples into principal components and describing the relationship between each variable and allowing visualization of their relationship, i.e. whether they are similar or different. If they are similar, they are grouped together while different samples are further apart (Abdi and Williams, 2010). The so-called factor plots are often used to visualize the data (Fig. 2).

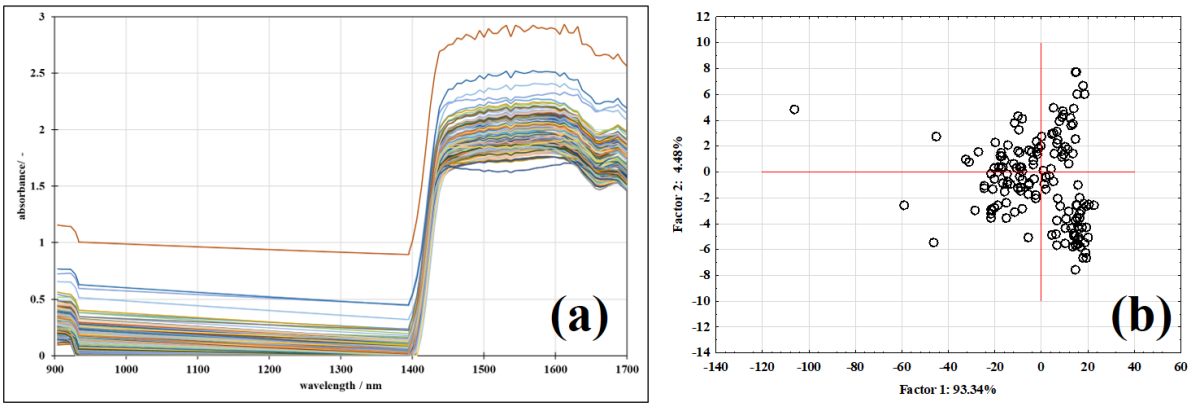

Fig. 2. (a) NIR spectra of honey samples (b) factor plot of NIR spectra samples

The values of the factor components for the spectra that differ are separated, while the values of the factor components for similar spectra are very close to each other, which allows their grouping (Jednačak and Novak, 2013). The goals of PCA are:

1. to extract the most important information from a set of data
2. to reduce the dimensionality of the data - by introducing principal components (PC), the dimensionality of the space is reduced, which simplifies the interpretation of the data. The first principal component (PC1) describes the largest variation in the data, while the remaining variation is described by other principal components (PC2, PC3, ...) (Jednačak and Novak, 2013).

Partial Least Squares (PLS) regression is a multivariate technique used to develop models for latent variables or factors (Djuris et al., 2013). The PLS method is used to describe the data using a model in which the smaller the number of variables, the better. Unlike PCA, where principal components are determined only by the variance of the measured variables and are independent of their correspondence with the desired property, in PLS the latent variables are selected to obtain the best correspondence between the property under study and the measured variable. PLS can be considered as a regressive form of PCA that combines data with a linear multivariate model located in two matrices, X and Y (Jednaček and Novak, 2013). The X and Y blocks (data sets) are modelled to find the variables in the X matrix that best describe the Y matrix. The information in the original X data is projected onto a small number of basic ("latent") variables to ensure that the first components are those that are most important in predicting the Y variables. The goal of PLS analysis is to describe the relationship between objects in X-space and their position in the corresponding Y-space with minimal deviations (Héberger, 2008).

Artificial neural networks (ANNs) are, in the broadest sense of the term, artificial replicas of the human brain that attempt to simulate the learning process and the role and function of the brain itself. Artificial neural networks consist of groups of interconnected elements called neurons. These neurons are organized into layers that form the "architecture" of the network. The first layer is called the "input layer", and each of its neurons receives information from the outside (generally independent variables are used as inputs). The last layer is the "output layer", which contains the neurons with the response or responses - depending on whether one or more parameters have been quantified. Layers of neurons between the input and output layers are called "hidden layers". Input and output data (X and Y matrices) are used to train the network, e.g. by changing the weights for each connection; the sum of all inputs for a given neuron transmits the information using an appropriate transfer function (e.g. sigmoidal function, tangens hyperbolic ...) and passes on the results (Héberger, 2008) (Fig. 3).

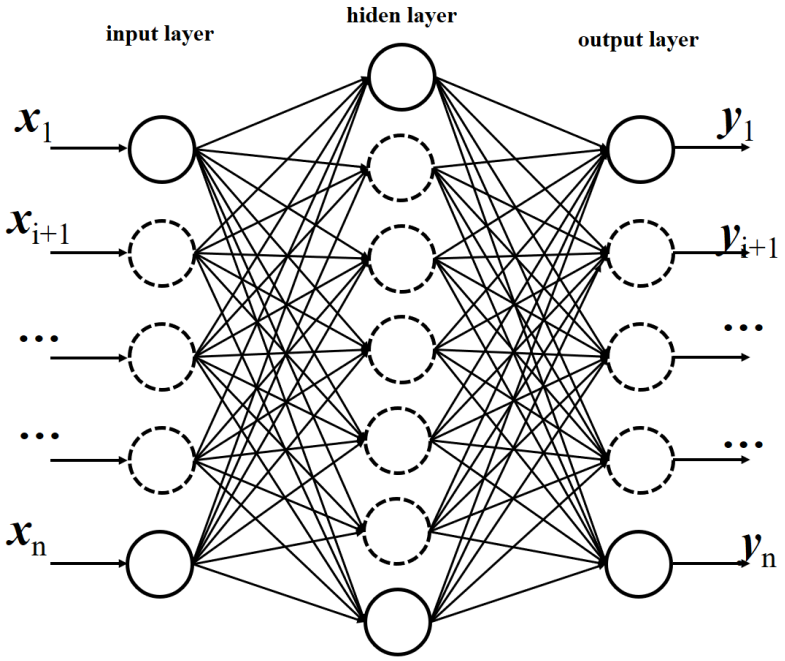

Fig. 3. General structure of ANN model

Neural networks with multilayer perceptrons consist of perceptron neurons organized in serially connected layers. The layers are usually described by numbers from 0 to N, where the zero layers only passes the vector of inputs to the network, while the N-th layer is also the output layer of the network. The layers between the zero and N-th layers are called inner or hidden layers. Multilayer perceptrons have the ability to learn. They also have an algorithm called the "Reverse Back Propagation Algorithm", with capabilities such as: initializing the weights of the network, displaying the first input vector, propagating the input vector through the network to obtain the output, calculating the error signal by comparing the actual output with the desired (target) output, propagating the error signals through the network, adjusting the weights to reduce the total error, repeating all steps with the next input vector as long as the total error is not satisfactorily small. Multilayer perceptrons are used for a variety of tasks that can be categorized as prediction, function approximation, or classification examples. Prediction is about predicting future trends in a time series of data given current and past conditions. Function approximation is about modelling relationships between variables. Sample classification is about classifying data into discrete classes.

Application of near-infrared spectroscopy in the detection of food adulteration

Authenticity of food and agricultural products is of significant importance to meet consumer demands and avoid unfair competition in the market (Downey, 2013). Food authenticity is an established area of research that involves the use of various analytical methods such as gas chromatography (GC), mass spectrometry (MS), nuclear magnetic resonance

(NMR), DNA fingerprinting and vibrational spectroscopy (NIR) (Downey, 2013). Although methods such as GC-MS can accurately detect the food adulteration, they have numerous shortcomings. They require highly skilled manpower, long and extensive instrumental analysis and complicated sample preparation. Therefore, there is an obvious need for the development of simple, rapid, non-destructive and cost-effective analytical methods for the detection and quantification of pathogens in honey. NIR spectroscopy is a rapid, very accurate, multi-analytical method based on the electromagnetic absorption of organic compounds in the short wavelength region of the infrared spectrum. It is used for qualitative or quantitative analysis of multiple sample constituents by a single measurement. Moreover, it does not require reagents and no hazardous waste is generated (Bázár et al., 2016).

Due to the growing problem of food adulteration, NIR as a simple, non-destructive method is everything that makes it more widely used in the chemical and food industries. Basri et al. (2017) in their study used MicroNIR (a palm-sized device) to find lard as a counterfeit component in palm oil. The results agreed with the mean square error of less than 1, which shows that the device has good repeatability. Vanstone et al. (2018) used NIR and related methods to detect adulteration of extra virgin olive oil with various vegetable oils. "Counterfeit" producers mainly use corn oil, sunflower oil, and soybean oil because they are the most accessible and economical. Oils like palm oil, however, are also much easier to detect. They have shown that NIR can distinguish the types of edible oil used for counterfeits and detect counterfeits in any sample with a low detection limit. Mendes et al. (2015) described the use of three different methods to detect counterfeit extra virgin olive oil with soybean oil. This work showed that NIR, in combination with certain chemometric methods, could be a useful alternative technique for the industry to identify possible adulteration in food products, as it is easy to apply and does not require specific chemical knowledge to perform measurements. Radman et al. (2018) applied NIR spectroscopy to detect gluten as a contaminant in food. The determination of gluten in food is very important because there are people who cannot tolerate it or are allergic to it. Two types of wheat flour were used to simulate cross contamination. Rice, rice flour, cornflour and corn semolina with a percentage of 5% to 30%. Based on the very satisfactory results of the study, the developed models could be used to predict possible cross-contamination with gluten. Mabood et al. (2016) tried to detect the adulteration of camel milk with goat milk using NIR. Camel milk is of great importance in desert countries as it contains high levels of vitamin C, up to six times more than cow's milk, and adulteration of this milk can human health. According to them, the newly developed NIR spectroscopic method combined with multivariate analysis is a suitable method to verify the detection and quantification of adulteration in camel milk.

Conclusion

From the above studies, it can be concluded that NIRs is a simple method in which there is no need for long-term sample preparation and which has excellent sensitivity and repeatability. In order to be on time observed and prevent food manipulations and questioning the authenticity of certain foodstuffs products, all the mentioned studies conclude that it would be desirable to encourage development in the future quick, simple and non-destructive methods. They emphasize that NIR could be one of the methods that should be further researched in order to develop as specific detection models as possible for their use in food adulteration detection.

Based on the findings of the above investigations, it can be stated that NIRs is a simple analytical method that requires no long-term sample preparation and has great sensitivity and reproducibility.

All of the research suggests that it would be beneficial to encourage the development of rapid, easy, and non-destructive procedures in order to be able to detect and avoid food manipulations. They emphasized that NIR is one of the technologies that should be investigated further in order to produce as specific detection models as feasible for application in food adulteration detection.

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