THE POTENTIAL USE OF NEAR-INFRARED SPECTROSCOPY FOR THE QUALITY ASSESSMENT OF EGGS AND EGG PRODUCTS

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Abstract
In a context of high productivity, eggs' quality assessment is necessary for enhanced safety and quality assurance towards the consumers and feedback for producers. The quality assessment of eggs and egg products is performed using destructive and time-consuming methods, therefore the use of rapid tools becomes mandatory, especially in the case of a high production rate. Near Infrared (NIR) spectroscopy is considered a very reliable and rapid technique with large use in food industry. At the farm level, NIR spectroscopy technique would be an interesting tool to determine the chemical and physical properties of eggs, eggshell and internal quality and, moreover, this information may help the layer farm manager when a problem occurs in the flock. Its application in the egg industry is aimed at the quality changes in eggs during storage and quality assessment of the egg products, through the compositional analysis. It is possible and in some cases successful the prediction and/or determination of different parameters such as: protein, total lipid and total solids content (for liquid egg products), polyunsaturated fatty acids (for freeze-dried egg yolk), moisture, fat and protein content (for spray-dried whole eggs). In addition, for the white colored shell eggs, the detection of blood and meat spots is also successful. Further studies with NIR and near infrared hyper spectral imaging system (NIR-HSI) are needed in this direction, as the results obtained until now are very promising for the development of a rapid tool for quality assessment of eggs and egg products.

***Key words**: chemometric tools, eggs and egg products, NIR, non-destructive methods, quality assessment.*

INTRODUCTION

Egg quality is a general term which refers to several standards defined by external and internal quality. External quality is focused on shell cleanliness, shell integrity and pigmentation and eggs shape, whereas internal quality refers to egg white (albumen) viscosity, lock of meat and blood spots, size of the air cell, yolk shape, strength and color. Internal egg

quality involves functional, aesthetic and microbiological properties of the egg yolk and albumen (Leeson, 2006).
Nowadays new technologies, fast, fully automated, reliable and nondestructive, such as near infrared spectroscopy, fluorescence spectroscopy, nuclear magnetic resonance, vibration analysis, or analysis of acoustic vibrations offer the possibility to evaluate the quality of a complete batch (control all the eggs from a lot), and not only a sampling (Mertens et al., 2010). Bamelis et al. (2004) mention that the availability of powerful computers and new detection technologies has enabled the development of fast, objective and accurate technologies.
In 2010, Mertens et al. presented several novel methods for eggs quality assessment, as it follows:

– near infrared spectroscopy: the measurement of the shell color as an indicator of stress and the general health of laying hens (the transmission color value linked to dynamic stiffness are related to less healthy hens possibly producing less strong eggs which are more sensitive to breakage);
– fluorescence spectroscopy: shell color and albumen quality;
– nuclear magnetic resonance or vibration analysis: eggshell integrity, eggshell strength;
– analyses of acoustic vibrations: albumen quality, fishy taint, shell integrity and dirt detection.

In this review we will focus on NIR spectroscopy technique because it ensures fast, low cost, reliable and non-destructive measures. The objectives of this paper are to describe the principle of NIR spectroscopy analysis, its advantages and disadvantages, the benefit for egg quality analyses by NIR spectroscopy. Finally, overviews of possible applications in egg industry, for quality assessment are presented.

NIR SPECTROSCOPY – general facts

Principle and theory

NIR spectroscopy is a vibration spectroscopic method very close to visible region, where the organic and inorganic compounds show good reference or transmission proprieties (Aenugu et al., 2011). Different systems such as visible-near infrared spectroscopy (VIS-NIR, wavelengths range: 450-1,100 nm), fourier transform-near infrared spectroscopy (FT-NIR, wavelengths range: 800-2,500 nm) or NIR spectroscopy (wavelengths range: 750–2,500

nm) reflect the absorbance of electromagnetic radiation at different wavelengths (Huang et al., 2008).
Near Infrared (NIR) spectroscopy is a well known technology which allows chemical information acquisition from samples (Rodriguez-Otero, et al., 1997). By NIR spectroscopy, C-H, N-H and O-H bonds are induced to vibrate. This principle is used to identify and quantify components. NIR spectroscopy allows the acquisition of the reflectance spectra of opaque milled or intact materials (Fig. 1).

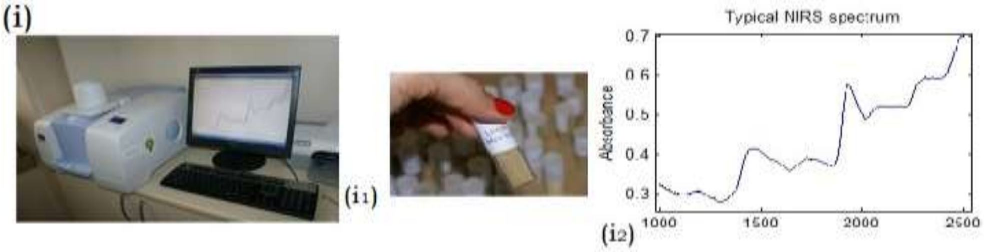

Figure 1. Acquisition of spectrum/spectra by conventional NIR spectroscopy system (source USAMV Cluj, Romania)

Legend: (i) NIR spectroscopy system (Forage Crops Lab, USAMV Cluj, Romania, Prof. PhD Rotar Ioan); (i_1) Sample support for NIR SPECTROSCOPY system; (i_2) Typical spectrum of NIR SPECTROSCOPY system.

NIR spectroscopy is characterized by the acquisition of a typical NIR spectrum which can be considered as the spectral signature or spectral fingerprint of the material. It is an adequate technique for the analysis of major components (chemical composition, detection and quantification of microorganisms) in agro-food products with minimum sample preparation.

Spectral analyses

Near infrared spectrum is recorded with the aim of getting information about the structure of a compound. In order to get efficient qualitative and quantitative information from data coming from NIR spectroscopy, chemometric tools are necessary further on (Roggo et al., 2005). Chemometrics is the science of extracting relevant informations from measurements made in chemical systems, using mathematical and statistical procedures (Massart et al., 1988). Qualitative and quantitative analyses by NIR spectroscopy usually require the application of calibration algorithms based on physicochemical measurements.

The building of calibration models starts with a spectral pre-processing treatment: after collection of spectra, it is necessary to perform a pre-treatment to remove high- or low-frequency interferences. Different types of pre-processing treatments are used: polynomial baseline correction, Savitzky - Golay derivative, Standard Normal Variate (SNV), mean-centering and unit variance normalization among others (Gowen et al., 2007).
After the spectral pre-processing treatment the calibration algorithms can be applied for classification and quantification. In order to analyse the data, many multivariate analytical tools are used, such as: principal component analysis (PCA), principal component regression (PCR), multi-linear regression (MLR), partial least squares regression (PLS), modified partial least squares regression (MPLS), partial least squares discriminant analysis (PLS-DA), linear discriminant analysis (LDA) (Gowen et al., 2007).
The spectral pre-processing treatments and calibration algorithms are extensively reviewed in the literature (Roggo et al., 2005; Wise et al., 2006).
Assessment of calibration performance through NIR calibration model performances can be characterized by several parameters: standard error of calibration (SEC) or standard error of cross validation (SECV). For acceptable calibration model performance, an independent set of samples is used to get the standard error of prediction (SEP) and the squared coefficient of correlation (RSQ), which are used to describe the NIR analytical error when analyzing samples of unknown quantitative composition (Hartmann and Buning-Pfaue, 1998).

Advantages and Disadvantages

Major advantages of spectroscopic techniques are: the ease of use, repeatability and reproducibility, reasonable start-up cost, non-polluting, non-invasive and non-destructive analyses and the possibility of online or directly in the field implementation. By NIR spectroscopy, an average of spectra for one sample can be recorded.
The disadvantages of NIR spectroscopy are: the relatively high price of instruments; the requirement of huge hardware speed; the necessity of calibration models for standardization; the possible presence of spectrum which do not contain chemical information like for example bad spectrum (ElMasry and Sun, 2010), such as spies (Dale et al., 2012).

EGGS AND EGG PRODUCTS QUALITY

Table eggs composition and factors related to quality

The avian egg is characterized through its destination, the one of vehicle of reproduction, but also through its importance as a food product (Karoui et al., 2009). The most important characteristics related to eggs quality are: its nutritional composition, the shell characteristics and the quality of the internal components (the albumen and the yolk).

Eggs' nutritional composition is characterized by a high content of proteins and lipids, being considered one of the most complete food product for the human diet. Moreover, it contains several vitamins and mineral elements that are regarded as essential nutrients. The shell is considered the most important physical barrier for preventing microbial entry, therefore cracking and abrasive damage to this barrier enhances the possibility for microbial penetration and may lead rapidly to spoilage of the egg (Shebuski and Freier, 2009). Its composition includes: approximately 94 % of calcium carbonate, small amounts of magnesium carbonate, phosphate and proteins, as part of the organic matter (Nys and Gautron, 2007).

The albumen nutritional quality resides in its protein content, of approximately 10 % (Powrie and Nakai, 1986). Its quality depends very much on the storage conditions, due to the known loss of CO_2 and the pH increase. The albumen becomes thinner and more transparent due to the aging process, which involves also a change in the protein conformation (Karoui et al., 2009). Jin et al. (2011) observed that during an extended period of storage, from 2 to 21 days, and due to an increase in temperature, from 5 to 21 and further on to 29 ^{o}C, the albumen pH increased (mainly due to temperature value), while the percentage of the albumen significantly ($p < 0.001$) decreased.

The yolk represents 33 % of the liquid weight, and its composition consists of fat (almost all the fat in the egg) and proteins. Also, liposoluble vitamins are present in its composition, with a highlight on vitamin D, egg yolk being one of the few food products naturally containing vitamin D. The pH of the yolk is 6.0 and during storage it remains constant, due to no CO_2 losses occurring (Karoui et al., 2009). Jin et al., (2011) showed that the yolk weight increases significantly during the storage, with increasing storage temperature and time.

Eggs are purchased and consumed as such, but at the same time, they are a very important part of the food industry, as they are extensively used as ingredients, due to the components' unique functional properties: foaming and gelling. For example, foaming is important in food industry for the production of bread, cakes, crackers, ice cream (Stadelman and Schmieder, 2002). As different changes occur during storage – the thinning of the albumen (Li-Chan and Nakai, 1989), the increase of pH value, weakening and stretching of the vitelline membrane and increase in yolk water content (Karoui et al., 2009) – their monitoring is very important for an immediate quality assessment. These changes depend mostly on storage time (Berardinelli et al., 2005), environmental temperature and relative humidity (Roberts, 2004), but also on hen age and strain (Silversides and Scott, 2001).

Table eggs and egg products and their quality indicators

UNECE standards for the certification and the control of shell eggs have been published in 2010. Concerning the grading of eggs, different parameters have to be observed, mainly regarding the physicochemical proprieties such as egg weight, shell quality (strength, color, cleanliness) and internal quality (freshness indicators).

The freshness is assessed through: air cell height (Sauveur and DeReviers, 1988) and Haugh unit measurements of the white (Silversides and Villeneuve, 1994). Storage temperatures as well as environmental conditions were the most significant factors influencing the quality of fresh eggs (Rossi et al., 2001). During storage, Kato et al. (1981) showed that a loss of CO_2 occurs from the egg through the pores in the shell, causing a thinning of the albumen. Also, Hill and Hall (1980) showed that the pH increases with the age of the egg, depending on the equilibrium between dissolved CO_2 bicarbonate ions, carbonate ions and proteins.

Rossi et al. (1995) proposed a new chemical index for the description of shell egg freshness, the determination of uridine ($C_9H_{12}N_2O_6$) and pyroglutamic acid ($C_5H_7NO_3$) concentrations. The index increase in the hen yolk or albumen is directly dependent on the storage temperature.

Considering the egg products, according to the UNECE standards for the marketing and commercial quality control of egg products (2010), these that are used nowadays in the food industry, are: the whole egg without shell (melange), liquid egg product, the egg yolk, the egg albumen, the frozen egg product, the dried egg product, the concentrated (condensed) egg product, the blended egg product, the fermented egg product, the stabilized egg product, the acidified egg product, the heat-treated egg albumen and the salted or sugared egg product (UNECE, 2010). Different indicators are used in order to assess the quality of conventional egg products: minimum solids matter content, minimum fat content, minimum protein content, extraneous matter, minimum concentration of hydrogen ions (pH), maximum beta-hydroxybutyric acid, maximum lactic acid, maximum succinic acid (UNECE, 2010).

NIR SPECTROSCOPY APPLICATIONS TO THE QUALITY ASSESSMENT OF EGGS AND EGG PRODUCTS

Nowadays, NIR spectroscopy is used from a large scale to a microscopic level. For this reason, new areas of work based on the NIR technology have been developed combining NIR systems and a microscope to create the NIR microscopy (NIRM) (Baeten et al., 2012). More recently, NIR was combined with imaging technologies creating the Near Infrared Hyperspectral Imaging System (NIR-HSI) (Fernández Pierna et al., 2004). Near-infrared spectroscopy technology offers the possibility to quickly and non-destructively analyze different constituents and properties of a product (Benson, 1995). Frequently hyperspectral imaging technique was used to detect omega-3 fatty acids in designer eggs, egg embryo development, differences between egg‐shell cracks and other egg‐shell features, or detection of hatching and table egg defects.

By NIR spectroscopy, diversified broad area of applications in eggs and egg products quality were carried out, revealing the possibility to use this technique in different ways, with a highlight on: whole egg and albumen quality, blood and meat spots detection and compositional analysis of egg products.

Whole egg quality assessment

Eggs' freshness

Together with shell integrity, the freshness is considered as one of the most important criteria to classify whole eggs.

NIR spectroscopy was used by Schmilovitch et al. (2002) for the estimation of egg freshness, using the transmittance mode (530-1130 nm). By applying PLS regression to the data, NIR can predict the number of days after

hatching, the air chamber size, the weight loss as well as the pH value. The determination coefficient (R^2) varied from 0.90 to 0.92.
The use of NIR spectroscopy by Norris (1996) for the assessment of egg quality during storage revealed changes in the spectral data, occurring immediately after the lay, but without any correlation with the internal egg quality. However, as Kemps et al. (2007) revealed later, there is a great variation between eggs of the same age, possibly due to measurement errors, egg-dependent effects and different times of the analysis. Therefore, Bamelis (2003) used Vis/NIR to monitor the quality changes in eggs that were stored at 18^{o}C for 21 days. The transmission spectra were acquired daily on egg samples, giving a total number of 16 spectra for each egg. The spectra were scanned between 500 and 800 nm, with an integration time of 250 ms. An increase around 674 nm (correlated positively with an increase of the storage time) and a decrease around 663 nm (correlated positively with a decrease in Haugh units) were observed.
Using FT-NIR spectroscopy system (MATRIX™-F, Bruker Optics, MA, USA), Giunchi et al. (2008) acquired diffuse reflectance spectra in the range of 833 to 2500 nm. After each spectral acquisition, the freshness parameters (air cell height, thick albumen heights and Haugh unit) were also destructively measured. The predictive models showed an R^2 value of up to 0.722, 0.789 and 0.676 for air cell height, thick albumen heights and Haugh unit respectively, therefore the diffuse reflectance FT-NIRS appears to be able to discriminate shell eggs during storage.

Albumen quality

The work of Bamelis (2003) continued through Kemps et al. (2006), through a record of the transmission spectra of table eggs stored for 0, 2, 4, 6, 8, 10, 12, 14, 16, and 18 days at 18^{o}C, with a relative humidity of 55 %. Through Vis/NIR spectra, intact eggs showed a large variation considering the proportional transmission values between eggs with a comparable albumen quality. Further on, a PLS regression was applied and the results showed that the correlation coefficient between the measured and the predicted Haugh unit values were 0.84 and 0.82 for the calibration and validation set, respectively, with better results for the pH. The most information regarding albumen transmission values was found between 570 and 750 nm, the team attributing these bands to Maillard reaction, with products inducing the formation of melanoidins (Burley and Vadehra, 1989).

Blood and meat spots detection

According to De Ketelaere et al. (2004) blood and meat spots are considered the most common defects found in eggs, these being able to influence the choice of the consumer. For their detection, candling is currently used; however this method is imperfect and costly. Candling accuracy for the blood and meat spots' detection is also varying from 20 to 90 %, therefore the use of spectroscopic techniques such as Vis/NIR is regarded as an improvement in this direction.

The first study of using spectroscopic techniques for detection of the blood presence in the eggs was a trial of Brant et al. (1953). They started by assuming that the calciferous shell of the egg absorbs all transmitted light under 550 nm, therefore only the 577 nm absorption peak could be used in detection of the presence of blood in the eggs. Using this technique, they obtained good results with an accuracy of 99.70%. Later, Gielen et al. (1979) suggested that a wavelength between 585 and 610 nm should be chosen. Karoui et al. (2009) mention that the ratio between the two values has been called "blood value" and the ratio was used as an index.

De Ketelaere et al. (2004) showed that there is a difference between the transmission spectra of eggs containing blood and eggs without blood. These authors divided the transmission at 577 nm by the transmission at a reference wavelength in order to correct the values for eggshell thickness, egg size and other non-haemoglobin-related characteristics. Based on unpublished data, Karoui et al. (2009) mention that a small amount of blood in the albumen could only be detected when part of it is diffused in the albumen, while very small blood spots are often not accompanied by dispersed blood, and, consequently, hard to detect. A factor that has a great influence on the success of the meat and blood spots' detection is the color of the shell. It has been demonstrated that the rate of blood detection in white-colored shell eggs is higher than in brown colored shell eggs. The basis of this difference is the protoporphyrin, the brown pigment of the eggshell that presents optical properties which are very close to those of haemoglobin. This shows a band located around 589 nm, very close to the absorption peak of the haemoglobin (577 nm).

Compositional analysis of egg products

The analytical methods used nowadays for the compositional analysis of the egg products, in order to evaluate their quality, are time-consuming. The use of NIR reflectance may improve this, by a better time management. NIR reflectance was used to determine the moisture, fat and protein in spray-dried whole egg (Wehling et al., 1988), the results showing a SEC of 0.15 %, 0.20 % and 0.28 % respectively, using a calibration based on three wavelengths. The authors concluded that the use of additional wavelengths is mandatory for a successful analysis of this product.

Dalle Zotte et al. (2006) used NIR reflectance to assess its ability for the prediction of physicochemical composition of freeze-dried egg yolk samples from laying hens fed with four different diets enriched with different sources of *n*-3 polyunsaturated fatty acids. The samples were analyzed using NIR spectra between 1100 and 2498 nm. The results showed an unsuccessful prediction of pH and cholesterol. However, the prediction of polyunsaturated fatty acids was accurate, with an R^2 value of 0.98 for the alpha-linoleic acid. Contrary to this, for the pH, R^2 value was 0.21, 0.75 for the crude protein, 0.81 for the dry matter, and 0.06 for the ash.

A new way for the use of NIR spectroscopy in the egg products industry is the prediction of the F_{70}^{10} parameter (Zardetto, 2005). Usually, thermal treatment causes a reduction in the nutritional value of the food, as a result of the Maillard reaction, which makes amino compounds biologically unavailable. The F_{70}^{10} parameter is then calculated with the purpose to compare different combinations of time and temperature for the thermal processing, in order to attain the initial aim (inactivation of foodborne pathogens) but also to maintain the highest nutritional value possible (Bigelow, 1921). Zardetto (2005) evaluated the thermal treatment of fresh egg pasta, using reflectance spectroscopy in the range 1000-2500 nm. The models predicted the F_{70}^{10} values with a standard error of 0.16 and a R^2 of 0.91. Therefore, the use of NIR spectroscopy as a rapid tool for the determination of this parameter could be successful.

Considering liquid egg products, their specifications lead to mandatory assessment of total solid content, therefore a rapid method is needed for current use. Osborne and Barrett (1984) measured the protein, total lipid and total solid contents, using NIR transmission. They obtained a R^2 of 0.96, 0.98 and 0.99 for protein, total lipids and total solid contents, respectively, compared with standard procedures. The researchers suggested that NIR could be successfully applied for the determination of these parameters.

The prediction of macronutrients is nowadays regarded as necessary for egg product manufacturers, therefore the accuracy of NIR in their prediction should be investigated further on. Buning-Pfaue et al. (2004) used the NIR reflection spectra on liquid shell egg samples and sample mixtures from yolk, egg white and water. Using PLS regression of the NIR spectra and physicochemical parameters, a good correlation was found for dry matter, crude protein, total fat, cholesterol and lecithin phosphate, with a R^2 higher than 0.98 (except for the crude protein, 0.79).

CONCLUSIONS AND FUTURE DEVELOPMENTS

NIR spectroscopy is a very extremely reliable, nondestructive and rapid technique for the prediction of quantitative and qualitative chemical and physical properties of many foods. Nevertheless, these techniques need to use chemometric tools, preprocessing treatment techniques and assessment of calibration models in order to extract the maximum of information available.

NIR spectroscopy and Vis/NIR spectroscopy can be successfully used for the assessment of source indicators of eggs and egg products' quality but further studies are needed. Moreover, the hyperspectral imaging system is an emerging technology for diversified applications in food quality and safety, which is able to determine the internal constituents of food products and is of prime importance in the food industry. It will be interesting to implement this technology with egg's sector.

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