VIS-NIR transmission for the assessment of internal egg quality

F.R. BAMELIS*, B.J. KEMPS, K. MERTENS, E.M. VERHOELST, B. DE KETELAERE, E.M. DECUYPERE and J.G. DE BAERDEMAEKER

Egg Quality and Incubation Research Group, Katholieke Universiteit Leuven, Kasteelpark Arenberg 30, B-3001 Heverlee, Belgium
***Flip.bamelis@agr.kuleuven.ac.be**

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Summary
Former research has pointed out that during the storage period of eggs, changes in transmission spectra could be measured. In the presented research, two detailed experiments are described. In the first experiment, the evolution of the transmission spectra during the storage of the eggs was investigated. In a second experiment, correlations between the transmission spectra and the classical egg freshness measurements (Haugh Units and pH) were investigated using the PLS analysis. From these experiments, it was concluded that the processes that force the Haugh Units to decrease and the pH to increase can be monitored directly by measuring the optical transmission in the visible range through eggs. Since this measurement technique is fast and non-destructive, it could form the basis of a new measurement technique to estimate egg freshness with possible use in and commercial grading machines and research.

Introduction
Optical measurements have been used for the quality determination of several kinds of products both at a laboratory scale as in online processes. Successful results were already obtained for determination of dry matter in onions (Birth *et al.*, 1985), proteins in wheat (Reyns, 2002), sugar content in apples (Lammertyn, 1997) and maturity of avocado (Schmilovitch *et al.*, 1999). Also for the measurement of fat content of meat and milk and the monitoring of the coagulation process of milk optical measurement techniques are available. Nevertheless, the application of optical techniques for quality determination of eggs is rather limited. Norris (1996) was the fist to use a NIR measurement technique to determine egg freshness. However, the storage time of these eggs was only a few hours. Later the applicability of optical techniques (Near-near-infrared transmission) to estimate egg freshness was demonstrated again by Schmilovitch *et al.* (2002) in an experiment that compares groups of eggs.

More recently, when measuring the VIS/NIR transmission spectra of eggs, a large variation was found. This variation could be divided in a time independent and a time dependent part. The time independent part was related with eggshell thickness, egg size and the protoporphyrin content of the shell. Concerning the time dependent changes, during the storage of eggs, the T673/T663nm ratio showed the most remarkable changes. This was supposed to be related with the internal changes in the albumen of the egg and hence these should be related with the changing freshness of the eggs (Bamelis, 2003; Bamelis *et al.*, 2003).

Since optical measurement techniques are non-destructive and take only 250 milliseconds, these may be of high interest for use in egg grading applications. Although not much research has been done on this topic, two applications are commercially available today. First, the presence of blood in consumption eggs is measured optically in commercial grading eggs, based on the T577/T610nm ratio with 577nm being the characteristic absorption wavelength of blood and 610nm being a reference wavelength (Brant *et al.*, 1953). Second, at transfer from setters to hatchers, the total transmittance of the egg is used as a yardstick for fertility. Moreover, research pointed out that the growing embryo can already be detected based on the T577/T610nm ratio from 96hours onwards (Bamelis *et al.*, 2002).

The objective of this work is to examine how the VIS-NIR transmission spectra of the intact egg can be used to predict the interior quality of the individual egg. This is done in two separate experiments. In a first experiment, the changes of the T673/T663nm ratio for individual eggs are examined profoundly. In a second experiment, changes in the total transmission spectra are investigated with advanced statistical tools and linked with destructive measurements of albumen quality parameters namely pH and Haugh Units.

Materials and methods

EXPERIMENT 1

147 uncracked white-shelled freshly laid eggs (Lohmann, Nijs, Dessel, Belgium) were used in the experiment. All eggs were stored for 21 days in a climatised room at 18°C. Except during the weekends, a transmission spectrum was taken daily resulting in a total of 16 spectra from each egg.

During the measurement of the transmission spectra, the egg was placed with its sharp end up on a foam ring, in which a collimating lens was attached (see Figure 3-6). From this lens, an optical fibre transported the light to an Avaspec-2048 (Avantes, Eerbeek, The Netherlands) spectrophotometer. Above the egg, a halogen lamp was placed. The spectrum of the lamp is rather uniform with a range from 500 to 880 nm. Before and after the measurements, the egg is protected from light and from the lamp heat by a metal shutter, which is placed between the lamp and the egg.

An integration time of 250ms was used and for each egg the average was taken of two measured spectra. A Labview-program was written to coordinate the measurements.

All measurements are compared to the transmission spectrum through a reference Teflon-block and corrected for electrical noise. Due to this comparison, the amplitudes in the spectra will be expressed as a ratio. In this work, these spectra are indicated by a letter T. After calculating the transmission rates, the spectra were smoothed by a Savitsky-Golay technique using a window of 30 measurement points and a second order fit.

To correct the spectra for inter egg differences as there are the eggshell thickness and the size of the egg, the spectra were normalised. These spectra are presented by T_n.

Statistical analysis

Since all eggs are measured daily, the optical measurements are dependent. For structured data sets like these, a linear mixed model for longitudinal data analysis has to be used. The advantage of this model is that it can handle non constant variance on the dataset. Moreover, the variance can be divided in two parts. The first part of this variance is the variance caused by the inter egg variance. The second part of the variance is caused by model misfits and lack of accuracy of the measurements. Calculations to estimate these parameters are done using the SAS 8.2 software package (SAS Institute Inc., USA).

EXPERIMENT 2

During the experiment a total of 600 intact white-shelled Lohmann® eggs (Nijs, Dessel, Belgium) were measured. To obtain a considerable variation in albumen quality, groups consisting of 60 eggs were stored for respectively 0, 2, 4, 6, 8, 10, 12, 14, 16 and 18 days. For each egg, a non-destructive spectral transmission measurement was done as described before. In stead of smoothing the spectra with a Savitsky-Golay approach, moving average was applied. Thereafter, the classical freshness parameters (Haugh unit and pH of albumen) were measured. To calculate Haugh Units of the albumen, the eggs were weighed (± 0.01g) and the albumen height was determined (± 0.25 mm) by a Futura® (Lohne, Germany) Haugh Unit measurement unit. Finally, the albumen was separated from the yolk and the pH of the thick albumen was determined by using a Schott® pH-meter (type CG840) after calibration of the pH electrode using buffer solutions of pH 7 and 10.

The transmission spectra measured in this experiment needed to be processed further. To correct them for the variable shell characteristics and egg size, full MSC (multiplicative scatter correction) is performed in the Unscrambler® software (version 7.8, Camo process AS, Norway). For this type of MSC both the amplification effect as the offset effect are removed from the spectra, which avoids that they dominate the information in the spectra.

Statistical analysis

After these pre-processing steps, the spectral data are linked to both Haugh unit and pH of the albumen by means of a PLS (Partial Least Squares) multivariate analysis. Partial Least Squares analysis of the samples was performed in the Unscrambler® software. Validation of the obtained models was performed by means of a cross validation. Examination of the residuals revealed that the model assumptions, i.e. linearity and constant variance, are fulfilled in case the Haugh unit serves as the dependent variable in the PLS analysis. For pH, on the contrary, a transformation of this variable was needed in order to meet the model assumptions. An inverse logarithmic transformation was needed to fulfil linearity and constant variable. Since the variable pH is proportional to the logarithm of the concentration of hydrogen, this transformation seems to be acceptable.

Results

EXPERIMENT 1

In Figure 1 left, the mean evolution of the T_n674/T_n663 nm ratio is shown. The same quadratic increase with storage time of this ratio was found in former research. In the right Figure, the variances on these mean values are presented. According to the linear mixed model for longitudinal data analysis, it was possible to divide this variance into the inter egg variance and the variance due to model misfits and lack of accuracy of measurements. As can be seen, there is an important increase of the inter egg variance with increasing storage time.

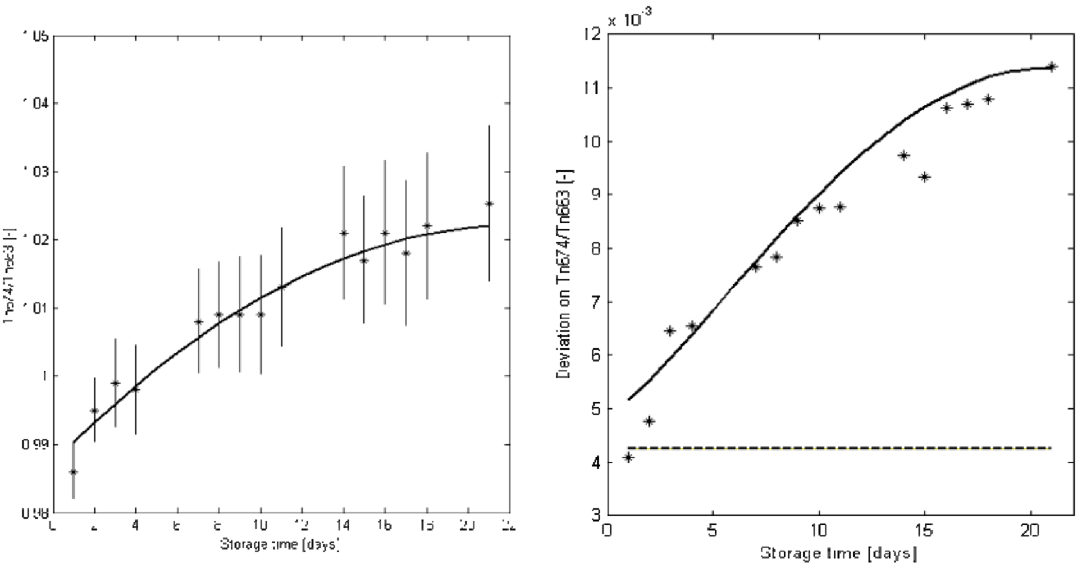

Figure 1 (left) Quadratic model between the storage time and T_n674/T_n663 nm ratio. Stars indicate the daily mean. Standard deviation for each day is given as an error bar. R^2=0.60, P<0.0001.

Figure 1 (right) Standard deviation the T_n674/T_n663 ratio. Standard deviation of the measurements is presented by *. The full line presents total deviation as calculated by the proc mixed mode ($\sqrt{Var(S_i)}$). The dashed line is the deviation due to misfitting of the model and measurement inaccuracy($\sqrt{\varepsilon}$). The zone between the dashed and full line equals the inter egg deviation.

In the next step, a quadratic function was fitted on the evolution of the T_n674/T_n663nm ratio for each egg separately according to equation 1. Hence, for each egg a b_0, b_1 and b_2 value was estimated. The b_1 and b_2 values were found to be highly correlated (R^2=0.82). Therefore, b_2 value can be estimated from b_1. Hence, the evolution of the T_n674/T_n663nm ratio can be characterised by one variable: b_0. The b_0 value is a constant value and was replaced by the mean value found in the experiment (0.987). The resulting model is presented in equation 2 and plotted in Figure 2. A correlation of 0.94 is found between the measured T_n674/T_n663nm ratio and the estimated values based on the b_1 value that is a characteristic for each egg in the experiment.

$$\frac{T_{n674}}{T_{n663}} = b_0 + b_1\,t + b_2\,t^2 \qquad \textbf{Eq. 1}$$

$$\frac{T_{n674}}{T_{n663}} = 0.987 + b_1\,t + (2.30x10^{-5} - 2.84x10^{-2} b_1)\,t^2 \qquad \textbf{Eq. 2}$$

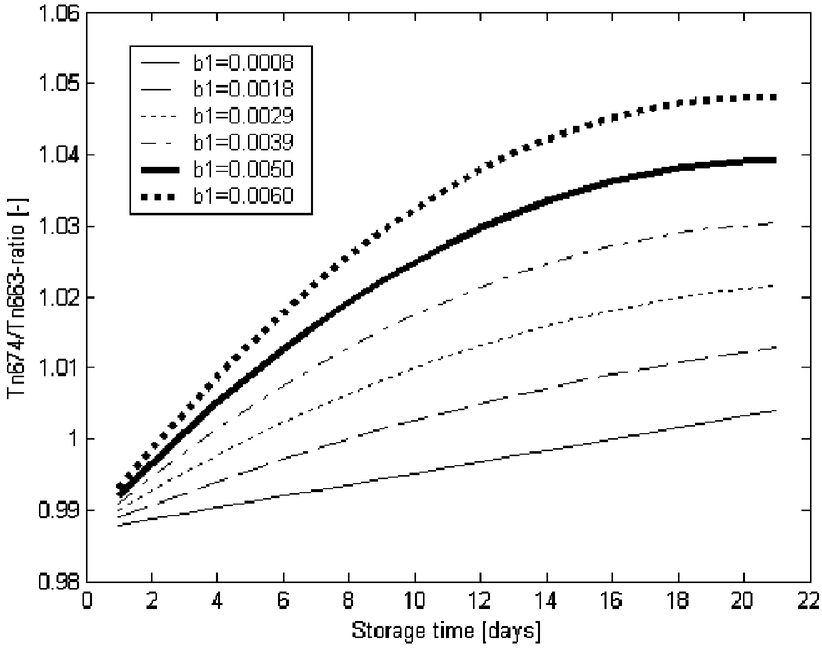

Figure 2 T_n674/T_n663 ratio in relation with storage time for different b1-values. Model is presented, the whole range of possible b1-values is used.

EXPERIMENT 2

A first indication concerning the influence of the albumen quality upon the transmission spectra of eggs can be obtained from Figure 3. Here, eggs with high, moderate and low Haugh Units and pH were grouped and the mean MSC corrected transmission spectra were calculated. These Figures suggest that the main changes in transmission spectra occur in the region between 500 and 750 nm. Around 630 nm the value of the MSC transmission increases as the egg ages. Around 655 nm, on the contrary, the value decreases. The same phenomena are observed for both pH and Haugh unit.

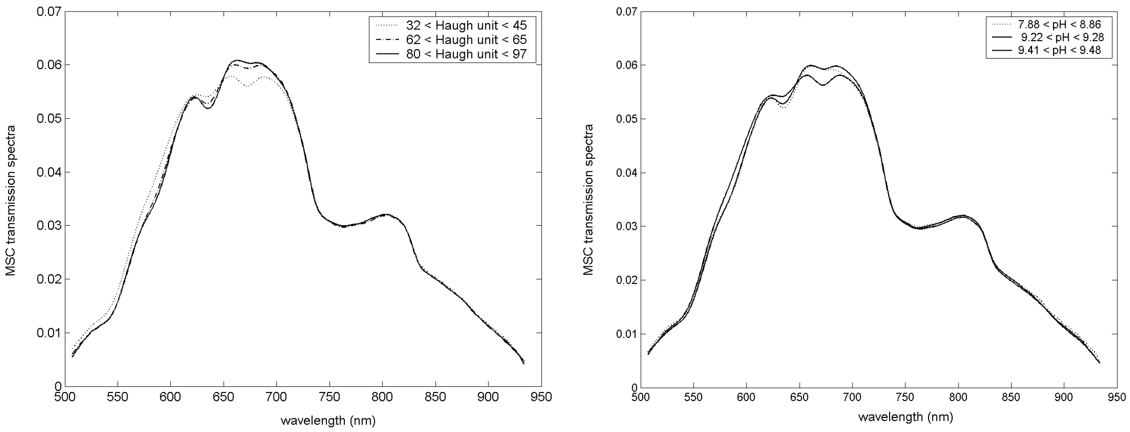

Figure 3 Mean MSC Transmission spectra for eggs with low, moderate and high Haugh Units (left) and pH (right).

During the PLS analysis the Haugh unit and a transformation of pH of the albumen served as dependent variables. The final model for both Haugh units and pH of the albumen was based on 4 latent variables. The correlation coefficient between the measured Haugh units and the prediction of the Haugh units based on the entire spectra was 0.842 and 0.816 for respectively the calibration set and the validation set whilst the correlation coefficient between the measured pH of the albumen and the prediction of the pH of the albumen was 0.867 and 0.861 for respectively the calibration set and the validation set. Figure 4 shows the predicted versus the measured values of respectively the transformed pH variable and the Haugh unit.

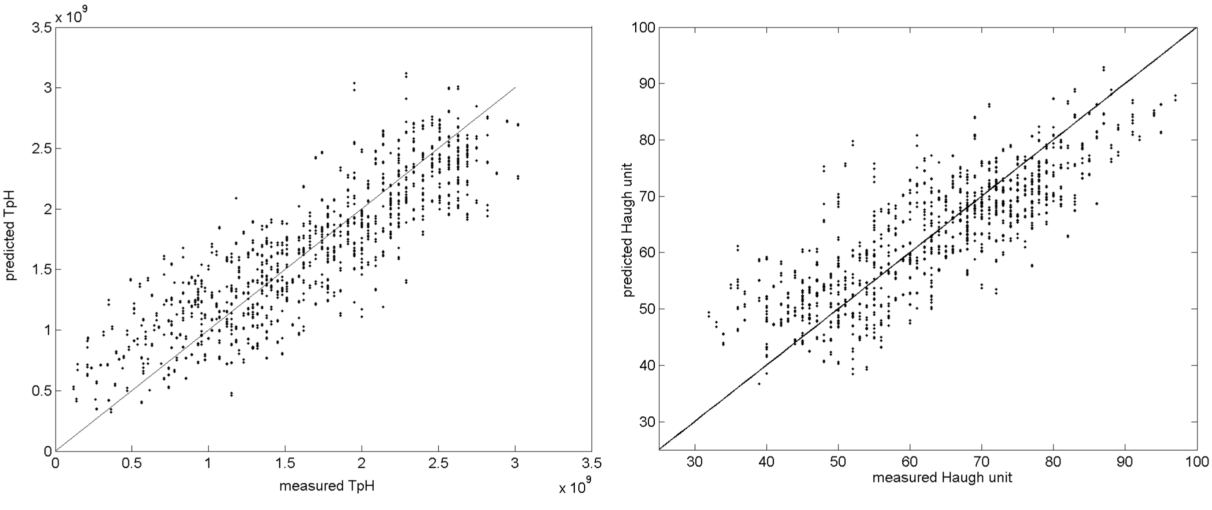

Figure 4 Correlation between the predicted values based on the PLS model and the measured values for Haugh Units (left) and pH (right) for each egg in the experiments.

An indication for the relative importance of the different wavelengths for the prediction of a variable can be obtained from the product between the regression coefficients and the standard deviation. For both the Haugh unit and the transformation of the albumen pH this product term is depicted in Figure 5. For comparison both curves were normalized to unity length. It appears that most information is obtained in the wavelength band between 570 and 750 nm. The similarity between the two curves in Figure 5 is remarkable: the shape of the curves is almost identical except that they are each other's mirror images. In fact, the correlation coefficient between these curves equals 0.986.

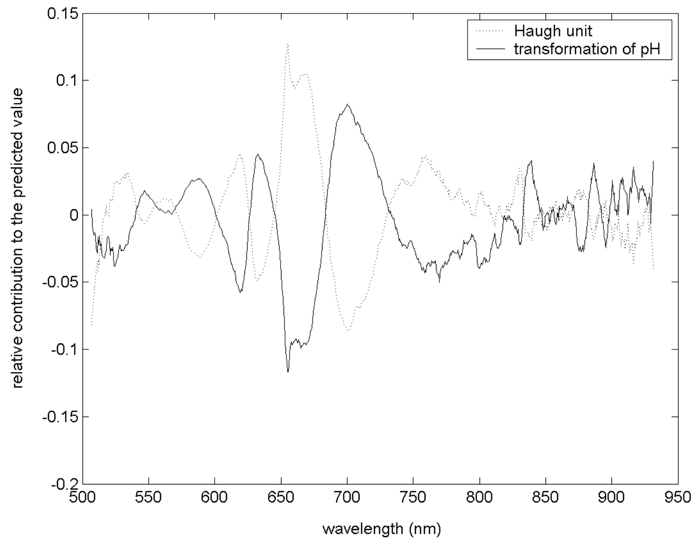

Figure 5 The relative importance of the different wavelengths for the prediction of Haugh Units (dashed line) and the transformation of the pH (full line) in the PLS models.

Discussion

From the first experiment, it is clear that an additional absorption peak is created in the transmission spectrum during the storage of eggs. This absorption peak is located at 663 nm (Bamelis, 2003). This means that the amount of an optic active substance (for example a specific protein) is increasing during the storage, what can be caused by an increasing amount of a certain substance or a change in conformation of a substance towards an optic active conformation. In the same experiment, it is shown that the process causing these changes is going on at a different rate in different eggs. The speed of this process is characteristic for each egg. The progress of this process, which can be monitored optically, might be an indicator for the decay of the eggs freshness.

In the second experiment described, both the pH of the albumen and Haugh unit of an individual egg were shown to be well predicted from transmission spectra. Though VIS/NIR spectra are obtained in this experiment, relevant information concerning egg freshness is restricted to the visible part namely between 570 nm and 750 nm. The inverse shape between the relative importance between the wavelength in the spectra and the transformed pH or the Haugh Units, can be explained by the fact that Haugh unit decreases during storage whereas the pH increases. Here, a strikingly high negative correlation (r = -0.986) was found between the curves shown in Figure 5. This proves that the physico-chemical processes driving changes in pH and Haugh are identical. Indeed, there exists a link between pH and Haugh unit through the physical interactions between ovomucin and lysozyme.

Until now, the molecules that cause the changes in transmission spectra are not identified. Presumably, the Maillard reaction or the browning reaction exerts an influence on the transmission spectra. This reaction, which is known to occur during aging, results in brown melanoidins (Burley *et al.*, 1989), which absorb light in visible part of the spectra.

As a conclusion, it can be said that the aging process in eggs can be monitored by measuring optical transmission spectra in the visible range in a non destructive way. These optical techniques are measuring directly biochemical changes, whereas the classical (and destructive) techniques are monitoring properties of the albumen indirectly influenced by the biochemical processes going on in the albumen. This might indicate that the optical technique not only has the advantage to be fast and non destructive and hence a useful tool for online egg grading, but also a more accurate tool compared with the classical tests for albumen quality.

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