

Albumen Freshness Assessment by Combining Visible Near-Infrared Transmission and Low-Resolution Proton Nuclear Magnetic Resonance Spectroscopy

B. J. Kemps,^{*1} B. De Ketelaere,* F. R. Bamelis,* K. Mertens,* E. M. Decuyper,*
J. G. De Baerdemaeker,* and F. Schwägelet

**Department of Biosystems, Katholieke Universiteit Leuven, B-3001, Belgium; and †Institute for Chemistry and Physics, Federal Research Centre for Nutrition and Food, D-95326 Kulmbach, Germany*

ABSTRACT Recently, some nondestructive methods for the assessment of albumen freshness were developed. Among others, visible near-infrared transmission spectroscopy and low-resolution proton nuclear magnetic resonance (LR ¹H NMR) measurements were proposed. This study was performed to evaluate the potential of the combined measurement of visible near-infrared transmission spectroscopy and LR ¹H NMR measurements for the assessment of albumen freshness. Our results show that

solely based on the transmission measurements, a good estimation of albumen freshness can be achieved. Based on LR ¹H NMR measurements, an estimation of albumen freshness can be achieved if larger egg collectives are used. However, when individual eggs are considered, only a moderate estimation is feasible. Finally, it was observed that combining both spectroscopic techniques did not improve the assessment of albumen freshness when compared solely to transmission measurements.

Key words: visible near-infrared transmission spectroscopy, low resolution proton nuclear magnetic resonance measurement spectroscopy, internal egg quality, albumen freshness

2007 Poultry Science 86:752–759

INTRODUCTION

Currently, there exist 3 major physical and chemical methods for the determination of albumen freshness. First, the height of the air space is used as a measure of egg freshness as it increases during storage. This increase is caused mainly by evaporation of water via the eggshell (Rossi et al., 1995). At this moment, the height of the air cell is the only quantitative egg freshness parameter considered by the European Union regulation. However, these values are not completely reliable, because the initial height of the air space varies substantially. For example, hen age, egg weight, and the prevailing environmental conditions exert an influence upon this parameter. Second, the pH of the egg white is another parameter that can be used to estimate albumen freshness. The rise in albumen pH during storage is caused by loss of CO₂ from the eggs through the pores of the shell. Albumen pH of an egg at oviposition has a value around 7.6 and increases to a value of about 9.5 during storage, after which it levels off or even slightly decreases after a long storage period (Lapao et al., 1999). The buffering capacity for pH changes of the albumen is weakest from pH 7.5

to 8.5 (Cotterill et al., 1959), which causes a rapid increase in pH during the first days of storage. Its increase is accompanied by the liquefaction of the egg white or the decrease of viscosity (Heath, 1977). Third, during storage, the viscosity of the egg white and the stability of the vitelline membrane decrease. These characteristics are used in different procedures for the determination of albumen freshness, in addition to the albumen index (Heiman and Carver, 1936) and Haugh units (Haugh, 1937). The Haugh unit is a commonly used freshness parameter and is based on both the weight of the intact egg and the thick albumen height of a broken egg. When a fresh egg is carefully broken onto a smooth, flat surface, the yolk is in a central position surrounded by thick albumen. When an older egg is broken, the yolk is often displaced to 1 side, and the surrounding thick albumen has become thinner, resulting in a large albumen area. This results in a decreased albumen height, which consequently leads to a decreased Haugh unit. Furthermore, a variety of chemical changes occur in egg components, which can be used as indicators for egg quality. For example, aggregated ovomucin is dissociated into α - and β -ovomucin during storage (Miller et al., 1982), and ovalbumin is transformed into S-ovalbumin (Smith and Back, 1962).

Except for air cell height, all of the above-mentioned methods require the destruction of eggs. Recently, an extensive review concerning both destructive as well as nondestructive techniques was described by Kemps et al.

©2007 Poultry Science Association Inc.

Received May 3, 2006.

Accepted December 2, 2006.

¹Corresponding author: Bart.Kemps@biw.kuleuven.be

(2006). Concerning the present study, we focused on both visible near-infrared (VIS-NIR) transmission spectroscopy and low-resolution proton nuclear magnetic resonance (LR ^1H NMR) measurements. Both methods were proposed as alternative fast and nondestructive methods for the determination of albumen freshness. Schwägele et al. (2001) used LR ^1H NMR to determine the quality of intact eggs. For this technique, eggs are placed into a homogeneous magnetic field, and the H nuclei in the egg are transferred into an excited state by an electromagnetic pulse. Changes in the eggs were detected by calculation of the transversal relaxation times during storage. During the first week of storage, the transversal relaxation time showed an exponential decrease for all storage temperatures. Afterwards, the transversal relaxation time decreased linearly, particularly at higher temperatures. This could be due to increased liquefaction of albumen during storage. Laghi et al. (2005) also performed a proton nuclear magnetic resonance relaxation study of hen egg quality. They concluded that the change in the transverse relaxation in thick egg albumen results from an increase in the protein exchange rate resulting from a pH increase attributed to loss of CO_2 by diffusion through the eggshell.

Recently, Kemps et al. (2006) assessed the potential of VIS-NIR transmission spectroscopy to determine albumen freshness. To obtain a considerable variation in freshness, groups consisting of 60 eggs were stored (18°C , 55% RH) for 0, 2, 4, 6, 8, 10, 12, 14, 16, and 18 d, respectively. The nondestructive spectral measurements were compared with the 2 most widely used destructive freshness parameters, namely Haugh units and albumen pH. A partial least squares (PLS, type 1) model was built to predict Haugh units and pH of the albumen based on the transmission spectra, respectively. The correlation coefficient (R) between the predicted value and the measured value equaled 0.842 and 0.867 for Haugh unit and pH of the albumen, respectively. Their results show that the visible region of the light transmission spectrum of an egg provides quantitative information about egg freshness and that the near infrared region is of minor importance in the prediction of egg freshness. The objective of the present study was to investigate whether a combination of VIS-NIR transmission spectroscopy and LR ^1H NMR could improve the assessment of albumen quality when compared with both methods separately.

MATERIALS AND METHODS

A total of 310 intact, white-shelled eggs of the same flock (Lohmann Selectic, 210 eggs at 59 wk of age, 100 eggs at 60 wk of age) were used for the measurements. To obtain eggs with different albumen freshness, the eggs from the 59-wk-old hens were divided into 3 groups of 70 eggs each and stored at 15, 20, and 25°C and a RH of 60% for 1 wk. Another 100 eggs from hens of the same flock were purchased 1 wk later. Before the measurements were performed, the eggs were stored at 20°C for 2 h to exclude temperature effects in the measurements.

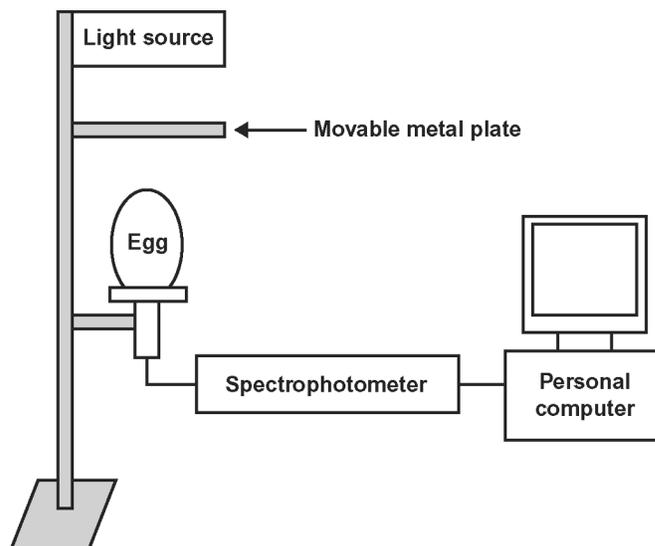


Figure 1. Experimental setup for the measurements of transmission spectra.

First, LR ^1H NMR spectroscopy was performed, giving rise to the transversal relaxation times after a 90° [T2(1)] and 180° [T2(2)] electromagnetic pulse. Besides these parameters, the amplitudes of the relaxation times are determined, which leads to the parameters AT2(1) and AT2(2), respectively. These parameters were determined on the intact eggs using a Minispec mq 10 NMR analyzer (Bruker, Karlsruhe, Germany). Subsequently, the VIS-NIR transmission spectrum was recorded for the same eggs. After these nondestructive measurements, Haugh units of the eggs were determined. For this purpose, the eggs were first weighted (± 0.01 g). Next, eggs were cracked, and the albumen height was determined (± 0.25 mm) by a vertically mounted micrometer (Futura, Lohne, Germany) connected to an electronic path. Based on both parameters, the Haugh unit is calculated (Haugh, 1937). The next 2 sections describe, respectively, how the VIS-NIR transmission spectrum is measured and how the relaxation times T2(1) and T2(2) are determined by LR ^1H NMR. Finally, the last section describes the statistical analysis being performed.

VIS-NIR Transmission

Figure 1 shows the experimental setup used for the measurements of the transmission spectra. The light source (halogen spot, 150 W; Osram, Capelle a/d IJssel, Belgium) is positioned above the egg. The egg is placed on a foam ring with its blunt end pointed up. The vertical placement of the egg leads to a better signal-to-noise ratio when compared with a horizontal placement. An optical fiber is placed underneath the egg and transports the transmitted light into the Avaspec-2048 spectrophotometer (Avantes, Eerbeek, the Netherlands). In this way, only light that passes through the egg reaches the collimating lens underneath the egg. The spectrophotometer has an optical range from 200 to 1,100 nm, with a resolution of

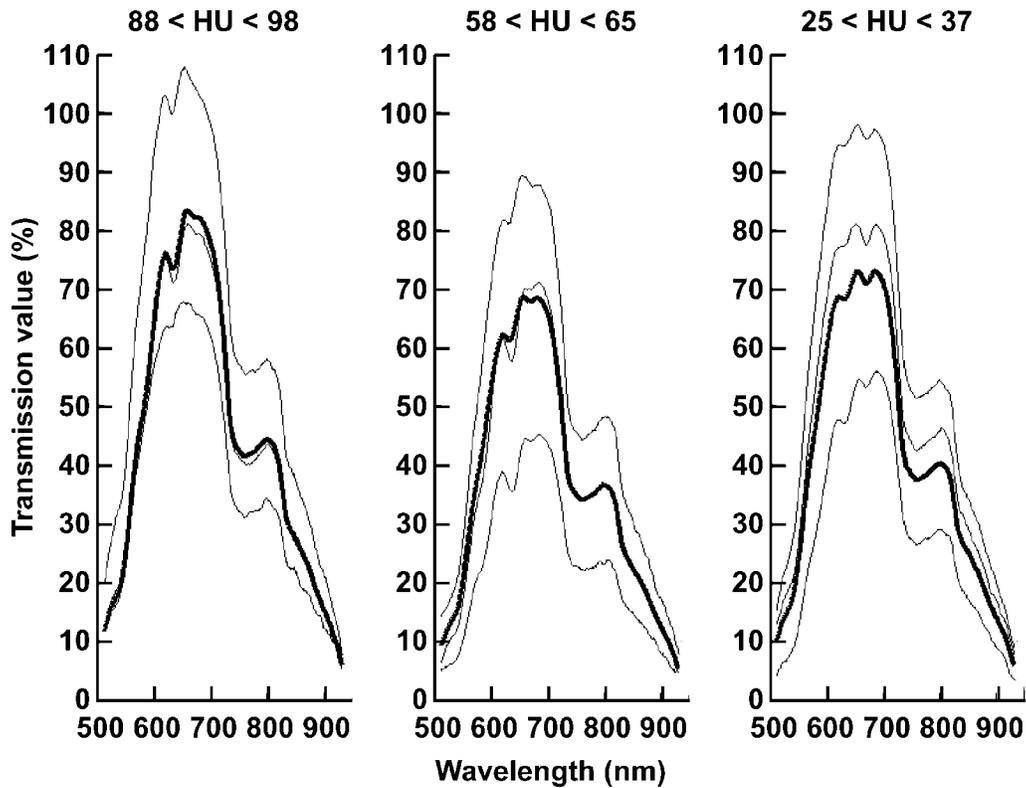


Figure 2. Proportional transmission spectra for 3 groups having distinct characteristics with respect to Haugh unit. The groups consist of eggs with the highest (left), average (middle), and lowest (right) values of Haugh unit (HU), respectively. For each group, the average spectrum (thick line) is given together with 3 individual spectra.

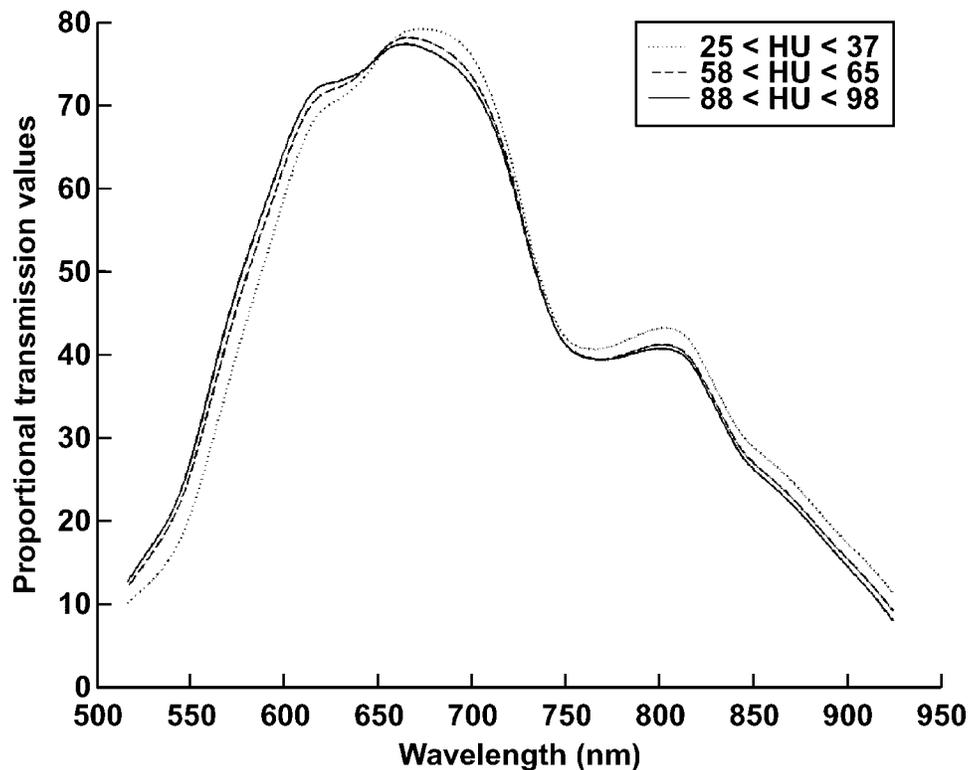


Figure 3. The mean multiplicative scatter correction transmission spectra for 3 groups having distinct characteristics with respect to Haugh unit. The number of eggs in each group equals 10% of all eggs measured. The groups consist of eggs with the lowest, average, and highest values of Haugh unit (HU), respectively.

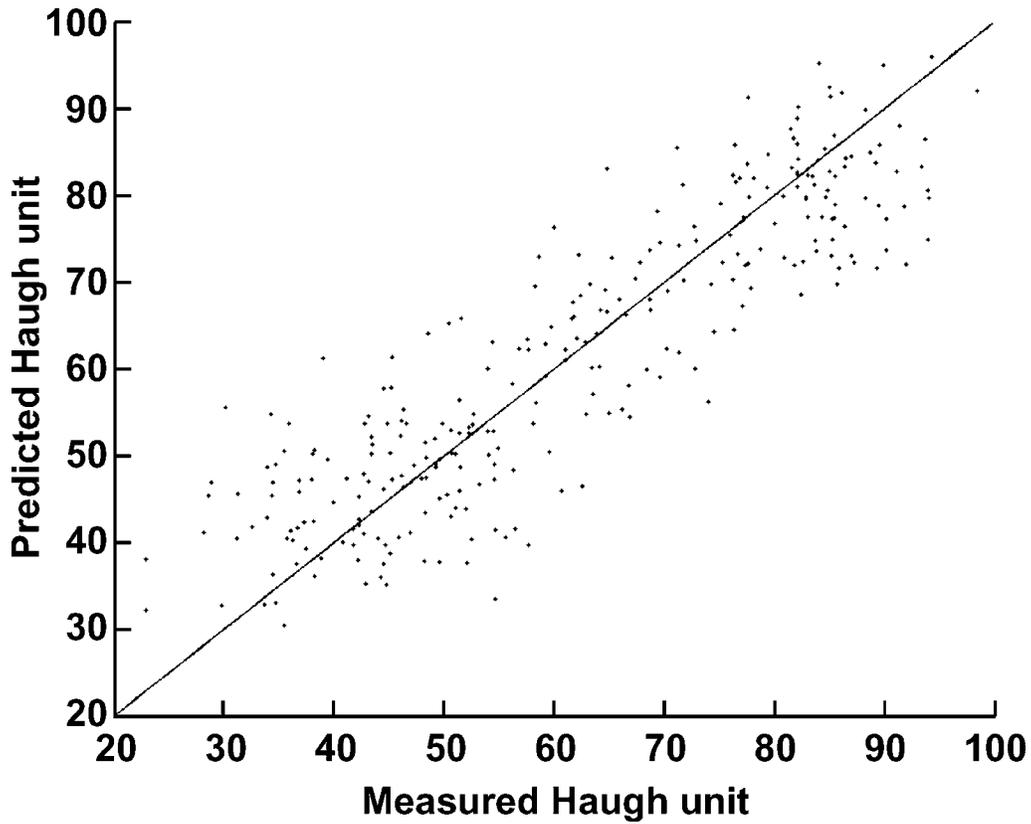


Figure 4. Predicted vs. the measured values of the Haugh unit based on the visible near-infrared transmission spectroscopy transmission model.

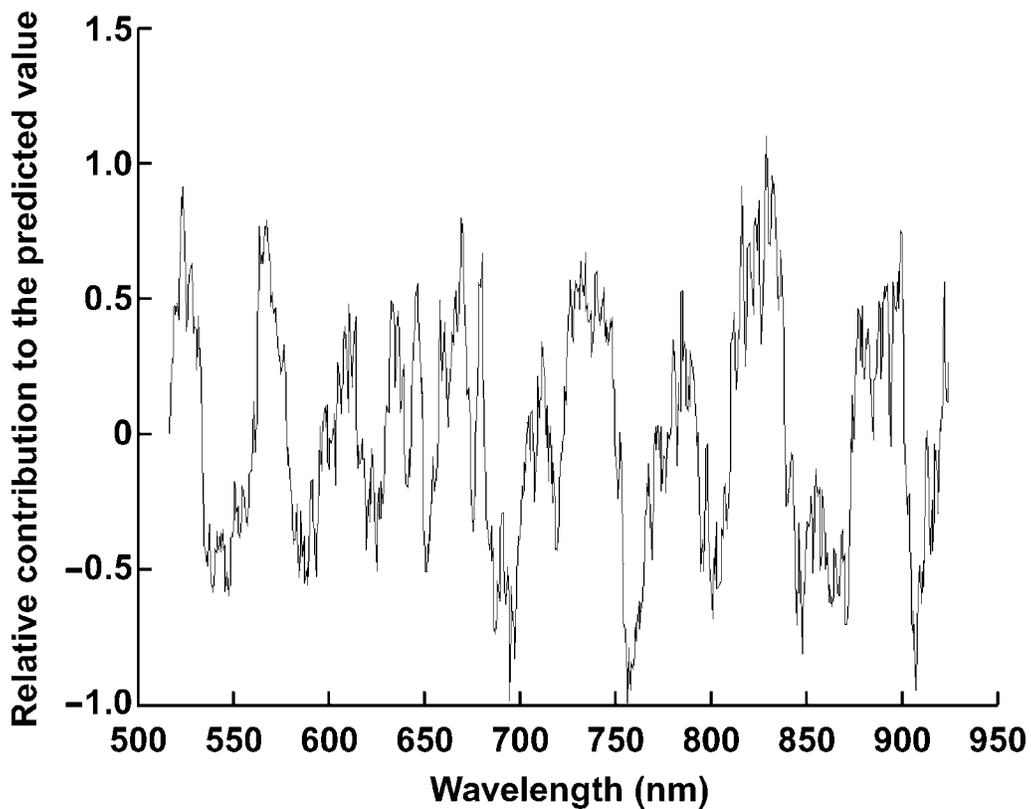


Figure 5. Plot of the relative contribution of each wavelength to the predicted value of Haugh unit.

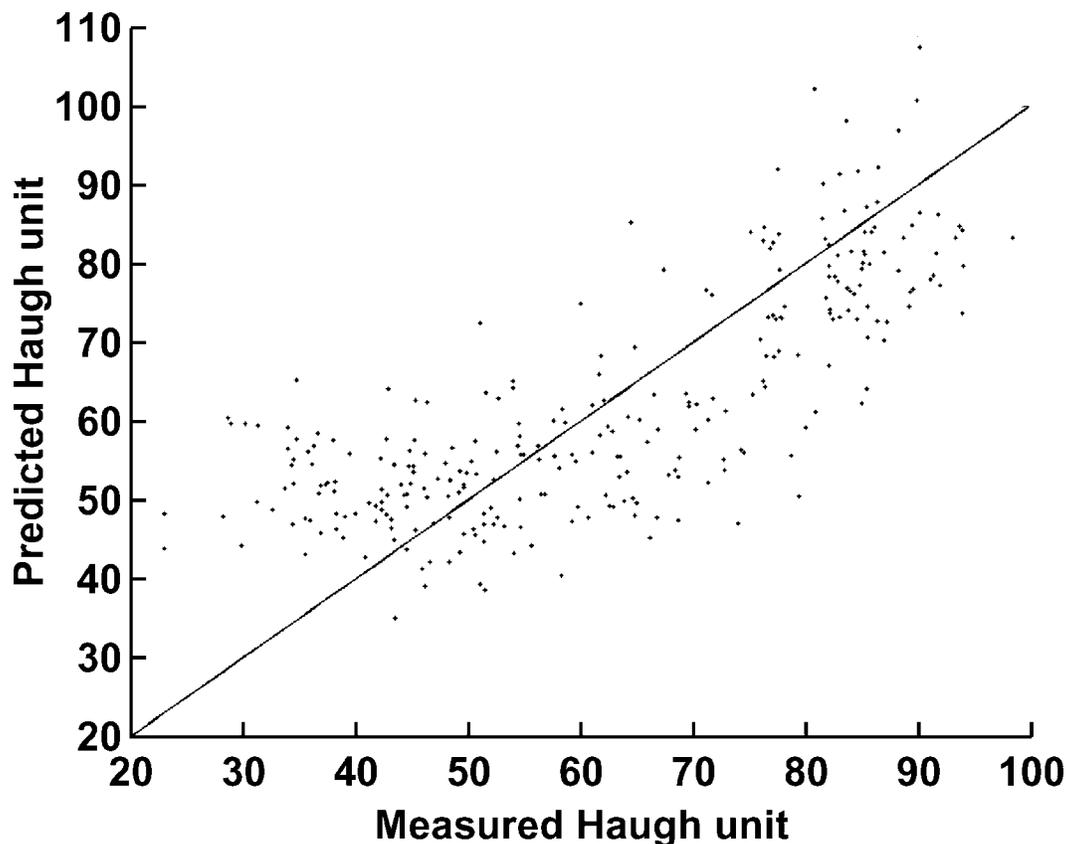


Figure 6. Predicted vs. the measured values of the Haugh unit based on the low-resolution proton nuclear magnetic resonance measurement spectroscopy model.

0.38 nm. To prevent warming up of the egg, a metal plate is positioned between the lamp and the egg. During the measurements, the plate automatically turns aside. Integration time for 1 measurement takes 250 ms, and the spectra of the eggs are obtained as the average of 2 subsequent measurements. To incorporate the changing characteristics of the light source, the spectrophotometer is calibrated before each measurement using a Teflon block (Bayer, Leverkusen, Germany) of 15-mm thickness. Because light is only scattered and not molecularly absorbed when it passes Teflon, the transmission spectrum of Teflon is nearly flat in the visible and near-infrared range (400 to 2,000 nm). This makes it suitable as a reference measurement. Furthermore, electrical noise is measured by taking the spectra when there is no light exposure to the spectrophotometer.

The raw transmission spectra of the eggs are transformed into the proportional transmission values (*P.T.*) at all wavelengths by the following formula:

$$P.T. = \frac{\text{raw transmission signal} - \text{electrical noise signal}}{\text{reference transmission signal} - \text{electrical noise signal}} \cdot 100\%$$

A Labview program (version 5.1, National Instruments, Austin, TX) was written to coordinate the measurements of the spectra and to calculate the proportional transmission values. Following the measurement of the spectra,

preprocessing is performed on the proportional transmission spectra. First, the spectra are smoothed by a moving average with a 50-points window. The proper size of the window was determined empirically. Kemps et al. (2006) mentioned a large variation in proportional transmission values of eggs with a comparable albumen quality and indicated that the total amount of energy that passes through an egg differs substantially irrespective of the albumen freshness of the egg. The panels of Figure 2 show some smoothed proportional transmission spectra of eggs having comparable albumen freshness. This variation is mainly caused by differences in egg size and characteristics of the shell (Vandeginste et al., 1998) rather than differences in albumen quality and indicates the necessity of a spectral preprocessing technique to examine albumen quality. Therefore, full multiplicative scatter correction (**MSC**) is performed in the Unscrambler software (version 7.8, Camo Process AS, Oslo, Norway). For this type of MSC, both the amplification effect and the offset effect are removed from the spectra, which avoids these dominating the information in the spectra (Vandeginste et al., 1998).

LR ¹H NMR

A LR ¹H NMR spectrometer Minispec mq 10 NMR analyzer (Camo Process AS) was used to determine transversal relaxation times T2(1) and T2(2) of intact eggs and

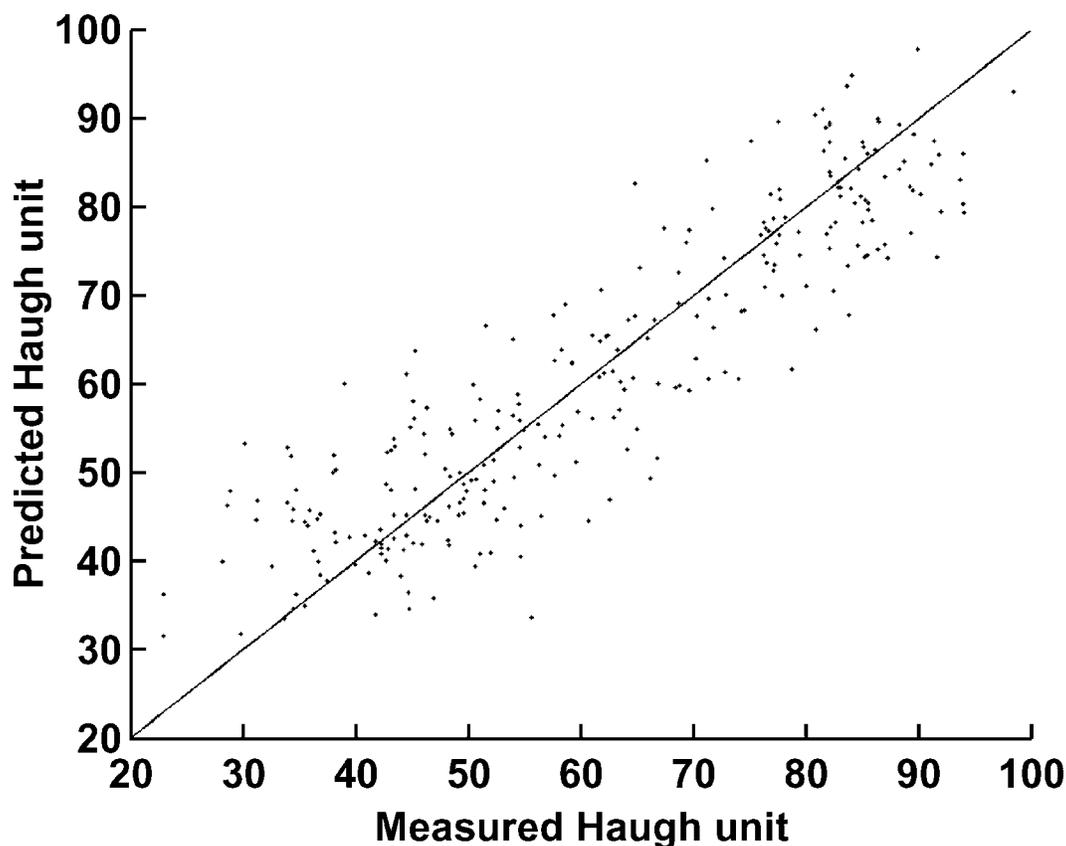


Figure 7. Predicted vs. the measured values of the Haugh unit based on both the visible near-infrared transmission spectroscopy transmission and the low-resolution proton nuclear magnetic resonance measurements.

their amplitudes, being AT2(1) and AT2(2), respectively. To perform LR ^1H NMR measurements, individual eggs are inserted into a homogeneous magnetic field using appropriate Teflon cuvettes. In this way, the H nuclei in the egg are excited by radio frequency pulses at 7.5 MHz. The resulting relaxation times T2(1) and T2(2) reflect the periods in which the magnetic activity is reduced to half its original value and are determined according to Bruker analytic software. For each egg, 4 scans of 20 s were made. The values T2(1), T2(2), AT2(1), and AT2(2) mentioned in the text were the average of 4 scans. For this application, a pulse length of 2 μs was chosen for the determination of T2(1) and T2(2).

Statistical Analysis

Three separate models were built to link the information obtained by the nondestructive measurements, with the destructive freshness parameter being the Haugh unit. The spectral data are linked to the Haugh unit by a PLS (type 1) multivariate analysis. Partial least squares analysis of the samples was performed in the Unscrambler software (National Instruments). To examine which information the parameters obtained by the LR ^1H NMR measurements contained about the albumen freshness, a multiple linear regression model was built. Multiple linear regression was performed using the stepwise procedure of the SAS software (version 8.2, SAS Institute Inc., Cary,

NC). Finally, it was investigated if the combined information of both the spectral and the LR ^1H NMR measurements could be beneficial. For this purpose, a PLS model (type 1) was built. Compared with the PLS model in which only the spectral information was taken into account, there were 4 additional independent variables being those obtained from the LR ^1H NMR measurements. Validation of the obtained models was performed by a cross-validation (leave-one-out cross-validation). Examination of the residuals revealed that the model assumptions, i.e., linearity and constant variance, are fulfilled for the above-mentioned models. Initial models were built on the complete data set. Outliers were detected manually by an examination of the X-Y outlier plot. This shows the relationship between the projection of the samples in the X-space and the projection of the samples in the Y-space.

RESULTS

VIS-NIR Transmission

A first indication concerning the influence of the albumen quality upon the transmission spectra of eggs can be seen in Figure 3. It shows the MSC spectra (full MSC, Unscrambler) for 3 distinct groups of internal quality based on Haugh unit. This figure suggests that changes in transmission spectra due to egg aging occur over the entire spectral region. Next, a PLS model was built based

Table 1. Final model obtained from a stepwise regression model of parameters obtained from the low-resolution proton nuclear magnetic resonance analysis

Variable	Partial R ²	Model R ²	F-value	P-value
T2(2)	0.5343	0.5343	323.48	<0.0001
T2(1)	0.0509	0.5851	34.44	<0.0001
AT2(2)	0.0066	0.5917	4.55	0.0338
AT2(1)	0.0059	0.5976	4.08	0.0444

on the spectral measurements and with the Haugh unit as dependent variable. Based on the X-Y outlier plot, 6 outliers were detected and omitted from the model. The final model was based on 9 latent variables. The R-value between the measured Haugh unit and the prediction of the Haugh unit based on the entire spectra was 0.888 and 0.870 for the calibration set and the validation set, respectively. Figure 4 shows the predicted vs. the measured values of the Haugh unit. The bisector line is also depicted in these graphs. An indication of the prediction capability can be obtained from the ratio of performance to deviation (RPD), which equals the ratio between the SD of the dependent variable and the root MS error of prediction (RMSEP; Starr et al., 1981). For the model based on the spectral measurements only, these variables equal 18.67 and 8.45, respectively. The RPD value equals approximately 2, implying that based on the VIS-NIR measurements, eggs can be subdivided into 2 groups with respect to their albumen freshness.

Information about which wavelengths contribute most to the prediction of the destructive freshness parameters can be derived from the regression coefficients of the PLS model. 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 SD of the spectral data. This product term is depicted in Figure 5. As indicated by Figure 3, information from 500 to 900 nm is needed for the prediction of the albumen freshness.

Finally, to investigate the influence of MSC upon the model, a comparable PLS model was built based on the smoothed spectra, i.e., not scatter-corrected. The correlation coefficients were comparable to the values of the MSC-corrected spectra. However, the RMSEP value of this model equals 9.80, indicating that prediction capability is considerably lower when the spectra are not preprocessed.

LR ¹H NMR

A multiple linear regression model was built with parameters obtained from the LR ¹H NMR measurements being the independent variables, whereas the Haugh unit served as the dependent variable. Stepwise regression was performed on a model containing the parameters T2(1), T2(2), AT2(1), and AT2(2) together with their quadratic terms and all possible interaction terms between the first-order parameters. Table 1 shows the final model obtained from the stepwise regression. The significance

Table 2. Comparison of different partial least squares models for the prediction of albumen freshness¹

Method	R	SD	RMSEP	RPD
VIS-NIR	0.888	18.67	8.45	2.21
LR ¹ H NMR	0.766	18.67	12.01	1.55
VIS-NIR + LR ¹ H NMR	0.899	18.67	8.85	2.11

¹RMSEP = root MS error of prediction; RPD = ratio of performance to deviation; LR ¹H NMR = low-resolution proton nuclear magnetic resonance measurement spectroscopy; VIS-NIR = visible near-infrared transmission spectroscopy.

level for parameter entry in the model was chosen at 0.05. The final model shows that both quadratic terms and interaction terms do not improve the model. Moreover, the parameters concerning the amplitudes and T2(1) only have a minor contribution in the prediction of albumen freshness. The R-value between the measured Haugh units and the prediction of the Haugh units-based final model was 0.773. Figure 6 shows the predicted vs. the measured values of the Haugh unit. The bisector line is also depicted in this graph. For the final model based on the LR ¹H NMR measurements only, the SD and RMSEP equal 18.67 and 12.01, respectively. The RPD value equals approximately 1.5, indicating that the prediction capacity is rather low.

VIS-NIR Transmission and LR ¹H NMR

In this case, both the parameters from the final LR ¹H NMR model as the transmission spectra served as the independent variables. Partial least squares analysis was performed in Unscrambler. In this case, 5 outliers were identified from the X-Y outlier plot. As for the model based solely on the spectra, the final model for Haugh units was based on 9 latent variables. The R-value between the measured Haugh units and the prediction of the Haugh units was 0.899 and 0.878 for the calibration set and the validation set, respectively. Figure 7 shows the predicted vs. the measured values of the Haugh unit. The bisector line is also depicted in these graphs. For the model based on both the spectral as the LR ¹H NMR measurements, the SD and RMSEP equal 18.67 and 8.846, respectively. The RPD value equals approximately 2, implying that with respect to albumen freshness, the eggs can be subdivided into 2 groups, namely fresh and old eggs. Finally, Table 2 summarizes the relevant parameters of the different models.

DISCUSSION

Based on the MSC transmission spectra solely, a good estimation of albumen freshness can be achieved. Williams (2003) stated that R² values from 0.66 to 0.81 point to approximate quantitative predictions. Because the coefficients of determination (R²) from the PLS model equal 0.78 and 0.76 for the calibration and the validation model, respectively, quantitative prediction of albumen freshness is feasible. Moreover, the regression coefficients of

the PLS model indicate that the spectral region from 500 to 900 nm is important in the prediction of albumen freshness. This result differs from the findings of Kemps et al. (2006), who stated that relevant information concerning egg freshness is restricted to the visible region from 570 to 750 nm. The difference in hen age, namely 45 vs. 59 wk of age, or flock differences could be pointed out as a possible cause for this observation. Further research is needed to clarify this inconsistency. Concerning the correlation coefficients and prediction capability of Haugh units, the values are comparable to the findings of Kemps et al. (2006), which validates these earlier results and provides further evidence to utilize VIS-NIR transmission measurements for the assessment of albumen freshness.

With respect to LR ^1H NMR spectroscopy, Schwägele et al. (2001) stated that T2(2) showed an exponential decrease for all storage temperatures and that this parameter therefore contains information concerning albumen freshness. In our work, the capability of LR ^1H NMR measurements to predict the internal quality of individual eggs was examined. The results show that based on LR ^1H NMR measurements only, a moderate prediction of albumen freshness is possible. The prediction capability of the LR ^1H NMR measurements are based almost solely on T2(2).

A plausible explanation for predominance of the VIS-NIR measurements concerning the prediction of albumen freshness could be the possibility to diminish interegg variations by the available preprocessing tools. The MSC applied in this work significantly improves the model. However, in the case of the NMR parameters, such a preprocessing tool does not exist, and the large interegg variation among eggs having similar albumen freshness consequently leads to a poor model.

Finally, the main goal of this work was to investigate whether the combination of both methods yields more reliable results concerning the estimation of albumen freshness of an individual egg. However, performing subsequently both measurements on the same eggs did not improve the prediction capability of albumen freshness when compared with transmission spectral measurements solely. Additionally, measuring the NMR parameters even slightly decreased prediction capability of the model.

ACKNOWLEDGMENTS

We thank the European community for their financial support (project EggDefence, QLRT-CT_2001-01732). Bart De Ketelaere and Flip Bamelis are postdoctoral fellows being supported by the Fund for Scientific Research-Flanders (FWO-Vlaanderen).

REFERENCES

- Cotterill, O. J., F. A. Gardner, F. E. Cunningham, and E. M. Funk. 1959. Titration curves and turbidity of whole albumen. *Poult. Sci.* 38:836–842.
- Haugh, R. R. 1937. The Haugh unit for measuring egg quality. *US Egg Poult. Mag.* 43:552–555.
- Heath, J. L. 1977. Chemical related osmotic changes in egg albumen during storage. *Poult. Sci.* 56:822–828.
- Heiman, V., and J. S. Carver. 1936. The albumen index as a physical measurement of egg quality. *Poult. Sci.* 15:141–148.
- Kemps, B., F. R. Bamelis, B. De Ketelaere, K. Mertens, T. Kokou, E. M. Decuypere, and J. G. De Baerdemaeker. 2006. Visible transmission spectroscopy for the assessment of egg freshness. *J. Sci. Food Agric.* 86:1399–1406.
- Laghi, L., M. A. Cremonini, G. Placucci, S. Sykora, K. Wright, and B. Hills. 2005. A proton NMR relaxation study of hen egg quality. *Magn. Reson. Imaging* 23:501–510.
- Lapao, C., T. L. Gama, and M. Chaviero Soares. 1999. Effects of broiler breeder age and length of egg storage on albumen characteristics and hatchability. *Poult. Sci.* 78:640–645.
- Miller, S. M., A. Kato, and S. Nakai. 1982. Sedimentation equilibrium study of the interaction between egg white lysozyme and ovomucin. *J. Agric. Food Chem.* 30:1127–1132.
- Rossi, M., A. Hildago, and C. Pompei. 1995. Classical methods and new proposals for rapid evaluation of shell freshness. *Proc. 6th Eur. Symp. Qual. Eggs and Egg Prod.*, Zaragoza, Spain. *World Poult. Sci. Assoc.*, Spanish Branch, Reus, Spain.
- Schwägele, F., R. Poser, and L. Krockel. 2001. Application of low-resolution NMR spectroscopy of intact eggs—Measurement of quality determining physical characteristics. *Fleischwirtschaft* 81:103–106.
- Smith, M. B., and J. F. Back. 1965. Modification of ovalbumin in stored eggs detected by heat denaturation. *Nature* 193:878–879.
- Starr, C., A. G. Morgan, and D. B. Smith. 1981. An evaluation of near-infrared reflectance analysis in some plant breeding programs. *J. Agric. Sci.* 97:107–118.
- Vandeginste, B. M. G., D. L. Massart, L. M. C. Buydens, S. De Jong, P. J. Lewi, and J. Smeyers-Verbeke. 1998. Data handling in science and technology. Pages 145–198 in *Handbook of Chemometrics and Qualimetrics. Part 20B*. Elsevier, Amsterdam, the Netherlands.
- Williams, P. 2003. Near-infrared technology—getting the best out of light. Nanaimo, Canada.