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NIR SPECTROSCOPY FOR RAPID FRESHNESS ASSESSMENT AND QUALITY CLASSIFICATION OF CHICKEN EGGS

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ABSTRACT

Eggs are the affordable and easy to available still mislabelling and wrong practice while selling the eggs is normal in the India or in the world. Egg freshness can be predicted accurately by using the proven methodology called Near Infrared (NIR) spectroscopy. Now a days affordable, and easy to handle technology is taking rise in food quality detection field which is basically in situ process. The main aim of this research was to provide low cost, fastest and on-site estimation of the freshness of the chicken eggs. The spectral analysis is based on the two parameters Haugh Unit (HU) and storage days which determines the freshness and quality respectively. To correlate the HU and storage days the partial least squares prediction model was developed and tested while its accuracy was the prime moto to evaluate by comparing predicted HU values with manually measured values. The developed model has a high correlation coefficient (R) of up to 0.98, showing a strong relationship between measured and predicted HU values. The model also shows the low root mean square error values (RMSEV) up to 1.119. This research concludes the scope of NIR spectroscopy as an important tool to detect the freshness of chicken eggs during storage period. The low mean square error value and high correlation assures the productivity of this non-invasive method to detect the egg freshness. The outcome ensures the effectivity of small, low cost and portable NIR spectrometer and project it as reliable device to measure the chicken egg quality. This research provides the vast scope for quality supply of eggs to the consumer. The results of the study can be compared with the other methodologies and are found better in predicting the egg freshness.

Keywords: *NIR, Freshness Detection, Eggs, Classification, Spectroscopy, Non-Destructive, Prediction Model.*

1. INTRODUCTION

The increase in the egg laying hen quantity and their requirement of better nutrition for their health causes substantial changes in the egg industry. A number of advancements occur in poultry industries by keeping an eye on the feed, nutrition and atmosphere of the egg lying hen. Though these developments have made it possible to produce eggs at a affordable cost, the poultry industry still must ensure that eggs fulfill consumer standards for quality [26]. The quality of an egg is measured by internal as well as external parameters. An egg's internal parameters are it's albumen and yolk quality. Albumen pH and HU of egg are often employed for evaluating these characteristics [13]. Traditional quality evaluation of an egg involves observation, candling and laboratory-based analysis, which is sometimes prone to error, time-consuming and requires skilled labor. That's why the poultry industry needs the non-invasive, cost effective, fast and accurate method to analyze the egg quality [24].

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Several non-invasive methods have been investigated to increase the egg quality measurement accuracy. All the investigated research is supposed to be prone to rapid and source-concentrated character of traditional methodologies while offering fair evaluations. The earlier technology used to predict egg quality was using the magnetic resonance imaging (MRI) which could evaluate the microanatomy of an egg [1], apart from this method there was another way to predict the yolk index by computer image analysis of an egg. There was the use of Electronic Nose (EN) [18] approaches also available which uses the smell sensors to find out the freshness of eggs. Furthermore, to estimate the egg white thickness the Near-Infrared Fourier transform (FT-NIR) spectroscopy has been used [4], indicating a appropriate measure of understanding relating spectral data and evaluated values.

The study was conducted in different phases from collecting chicken eggs from recognized poultry farm, then analyzing the different predictive models with various pre-processing techniques for NIR spectral data obtained for those collected chicken eggs. [11]. This study has multivariate chemometric analysis of spectral data.

This method could focus on progress in the estimation of egg superiority. We have used spectroscopic techniques, like Near-Infrared (NIR) spectroscopy [5], corresponding with enhanced data analysis approaches [22], after investigative the problems in the formerly defined non-invasive measures, to develop reliable and unbiased methodologies for estimating egg superiority.

The eggs are unfertilized before preserving them for spectral collection. The eggs are stored in a controlled atmosphere with constant humidity and two different temperatures i.e.20^{0}C and 30^{0}C. Spectra was collected for both the temperatures to analyze the effect of temperature on the HU and freshness index.

Rigorous preprocessing techniques were used on spectral data to reduce the effect of scattered lights and noise from it. It includes the scatter light correction, baseline corrections, smoothing for extracting the exact parameters without any kind of unwanted signals. Once the spectral data is pre-processed it undergoes the Principal Component analysis for reducing the multidimensional effect and finding out the principal components which show the maximum correlation [22]. These approaches were necessary for noise reduction feature selection, and data visualization, which made it easier to create dependable predictive models.

The pre-processed spectral data after principal component analysis was used for the regression analysis which was done by Partial Least Square Regression (PLS-R) model as well as Support Vector Machine Regression (SVM-R) model to predict HU value, a measure of egg freshness. Once the prediction was accurate, egg quality indicators were used to classify eggs using Partial Least Squares Discriminant Analysis (PLS-DA) [8].

Rigorous spectral analysis was done in this research and different pre-processing techniques were employed to enhance the spectral qualities before it's dimensionality reduction. This research proposes the precise preprocessing technique for bare minimum noise in the spectra before developing the prediction and classification model.

Largely, this research indicates that NIR spectroscopy coupled with different chemometric and multivariate analysis techniques can be effective tools for non-invasive estimation of egg superiority. Through the combination of modern spectroscopic techniques with robust data investigation tools, this research improves the conception of operational and balanced methods to evaluate the freshness of eggs and poultry industry quality.

2. LITERATURE SURVEY

A lot of research has been done for evaluating the egg parameters for grading them for their freshness indexes [2,16,9]. All the methodologies studied were extensively for non-invasive techniques focusing on internal parameters like albumen pH, HU, yolk index extraction to correlate them with the freshness. These methods involve image processing, Raman spectroscopy, infrared spectroscopy [6], front-face fluorescence spectroscopy [11], visible-near transmission spectra [5,8], and electronic nose-based systems [18].

Electronic nose-based approach has promising approach to determine the storage period of eggs by evaluating internal parameters. The

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method has extensively used genetic algorithms with backpropagation neural network to evaluate the internal parameters like yolk index and HU which estimates the freshness [18].

Another technique which has proven it's effectiveness is Visible-near transmission spectral analysis. It evaluates the egg freshness and quality, indicating substantial correlation coefficients for internal parameters such as HU, air cell height and thick albumen height. Likewise, visible transmission spectroscopy has been investigated as a non-destructive approach for evaluating individual egg freshness, generating superior correlation coefficients for calculating albumen pH and HU [6].

By considering all the approaches for their advantages and disadvantages the spectroscopy approach emerged as method which provide rapid, non-destructive technique with ensuring minimum physical contact during testing as well as evaluation was done with higher hygienic conditions. Spectroscopy reduces the extensive sample preparation requirement for prediction and grading the eggs.[9]

Furthermore, a study employing NIR spectral analysis has exhibited precise projections of several egg superiority factors, including storage days, albumen pH, air chamber height, albumen pH, and weight loss [16].

After studying all other techniques, we proposed the NIR spectroscopy as a non-invasive, rapid, affordable, on-line technique. This technique has the ability to precisely forecast multiple egg quality parameters. This technique has been selected for it's efficiency, accuracy, and potential widespread application in the poultry business. The methodology provides a rational solution for quality assessment, facilitating competent and dependable estimation deprived of the requirement for destructive testing or extensive sample preparation [7].

3. MATERIAL AND METHODOLOGY

The following methods would become employed to carry out the research:

3.1 Egg Sample Collection:

The eggs for the experiment were collected from different farms but of the same breed of hen and those hens were kept under the same atmospheric conditions and organically fed. A careful practice was followed to collect a total of six hundred and sixty (660) recently laid eggs, all precisely one-day old. These eggs had an average weight of 63.48 ± 2.56 grams, a height measuring 50.97 ± 1.53 millimeters, and a diameter of 37.34 ± 1.09 millimeters. The 330 eggs were sourced from each farm extensively non fertilized eggs were used for the experiment.

The 660 freshly laid eggs were carefully transported to the laboratory by their integrity. In the laboratory, they were disinfected by immersing them in 42°C hot water having 50 parts per million (ppm) of chlorine for a period of 1 minute. Afterward, the eggs were left to air-dry for 5 minutes at room temperature [1].

As previously mentioned, the collected eggs were stored after preparing them in two different temperatures. Those eggs were randomly divided for storage process under different temperatures. The first group was stored at 20°C, while the second group survived an different storage temperature of 30°C. These storage conditions covered intervals of 0, 4, 7, 10, 14, 17, 19, or 21 days inside a monitored chamber, keeping relative humidity between 50% to 65%.

For every testing interval, randomly selected 80 eggs (40 eggs from each storage temperature) were examined. Those eggs underwent a spectral collection method, and respective spectra of each egg were stored. The same eggs underwent destructive analysis for measuring the HU by calculating their yolk height and weight. Here in this study, we have considered HU as an evaluation parameter.

Precisely saying this study highlights on the effect of storage day and different temperature condition on the internal parameter of the chicken eggs which intern helped in evaluating the freshness of chicken egg.

3.2 NIR Spectral Data Collection:

The DLPR NIRscan™ Nano portable spectrometer, with a wavelength range from 902 to 1810 nm were used to collect the spectra of egg samples. This device is suitable for in situ measurement of spectra as it is portable. At the same time the device offers realism and flexibility, facilitating seamless data collection. The samples were collected for the interval of 4nm and operated in absorbance mode. We employed a 10-watt halogen lamp as the light source and an In GaAs sensor for sensitivity [22].

Each egg sample underwent spectral collection process for three different locations: R1,

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R2, and R3, which generated about a total of 1980 spectra in our database. To enhance the reliability of the model the spectral data matrix was created by averaging the spectra from different location such as (R1 + R2), (R1 + R3), (R2 + R3), and (R1 + R2 + R3), intended at evaluating the potential development in ensuing sorting and prediction models through a fusion of measurements from multiple locations [8].

Subsequently, we had seven databases, for training and testing prediction and categorization models, each database containing 660 spectra. This complete methodology granted us to explore intensely into the spectral tone of eggs, reflecting the influence of different locations and their impact on the performance of predictive models.

3.3 Evaluation of HU.:

The destructive analysis of egg samples was an important step for evaluation of predictive model. The HU was measured during the destructive analysis and equation (1) was applied to determine the HU for each egg, a system of measurement proposed by Haugh in 1937 [21]. This method involved precise measurements of egg weight and exact assessment of albumen (egg white) height. A high-precision digital scale was used to weigh each egg with an accuracy of 0.01 grams. Afterwards, the egg was gently cracked onto a glass plate, and using a vernier caliper with a resolution of 0.1 mm, the height of the egg white was measured three times around the yolk, approximately 10 mm from the yolk [10].

The HU was calculated using the following formula:

$$HU = 100 \log (h + 7.6 - 1.7w0.37) \quad (1)$$

Here,

h represents the average height of the egg white in millimeters, and w denotes the weight of the egg in grams.

3.4 Statistical Examination:

3.4.1 Spectra preprocessing:

The raw spectra collected at different locations by NIR spectrometer must undergo the pre-processing methods to reduce the [2] data dimensionality and enhance the reliability of the data while developing the model for prediction and classification. Number of preprocessing methods were employed to improve spectral data, lessen noise, and make the data ready for modeling. Preprocessing is employed by considering three basic factor light effect, baseline, and noise. All these factors should be treated while preprocessing the spectral data, or else model will have less accuracy. Prime important preprocessing phase is baseline correction, vital for removing systematic variations in spectral information produced by instrumental consequences like scattering. Common approaches for baseline correction contain linear or polynomial fitting to rectify baseline shifts.

The spectra initially treated to reduce the light scattering effect at different wavelengths. The Multiplicative Scatter Correction (MSC) or Standard Normal Variate (SNV) are employed to reduce the scattering effects [13]. The SNV treats the spectral data by achieving zero mean and unit variance while MSC is effective in balancing for multiplicative scattering, efficiently plummeting the effect of scattering effects.

Once the light scattering effect is reduced, smoothing techniques were employed to overcome the inherent noise and fluctuation problem. The Savitzky-Golay [13] methodology is used to reduce high frequency noise while retaining necessary information, confirming that the processed data is more responsive to subsequent modeling and analysis. The first derivate was employed to correct the baseline in the spectra. The mindful deployment of these preprocessing techniques substantially promotes the overall success and correctness of NIR spectroscopy in assessing chicken egg quality.

3.4.2 Estimation of HU:

To predict the HU, the well-suited prediction model for spectral data was developed using Partial Least Squares (PLS) regression technique. Pre-processed NIR spectra was used to derived response variables, for each individual egg based on the reflectance values. This approach aims to develop accurate prediction model for predictive HU by assessing the egg quality using spectral data. The regression model helps in enhancing our capability to estimate and preserve the quality standards of chicken eggs.

3.4.3 Egg categorization based on freshness ratings:

After predicting the HU of individual egg, it has been used for the classification process. In the

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study, we have employed Partial Least Squares Discriminant Analysis (PLS-DA) [9] for classification purposes. It is widely used approach for sample classification using chemometric analysis spectral data of the sample. During regression model analysis we had analyzed the importance of latent variables which describes the maximum covariance between the spectral data (predictor variables) and the class labels (response variables). Regression coefficients guide the involvement of each variable to the prediction of class membership. The pre-processing of the spectra is the part of the classification model where raw spectra undergoes the MSC, SNV for reducing the light scattering effect, S-G for reducing noise in spectral readings and S-G first derivate to correct the baseline in all spectral results. Principal components were used to optimize the model. The classification model was calibrated with a known class label. Validation and estimation measure the model's strength and performance metrics such as sensitivity, specificity, accuracy, and error rates.

The molecular changes in the chemometric analysis of the sample associated with the egg freshness. In this research classification of fresh and stale eggs was understood by relevance of spectral variations with respect to latent variables. PLS-DA provides the crucial characteristics for non-destructive classification model. This facilitates the assignment of the samples to their respective classes based on the chemometric analysis [8].For the classification model dataset must be distributed in two groups: Training and testing, the training dataset having 70% of the spectral data used to develop the model while testing dataset is of remaining 30% spectral data used to test that developed model. Partial Least Square Discriminant Analysis (PLS-DA) were executed to classify eggs according to their freshness. The classification model was tested for the tree classes (Class AA, Class A and Class B) and then by collective results of Class AA and Class A as fresh category and Class B as stale category. The suitable number of latent variables (LV) for regression and classification models was determined using the lowest root mean square error of cross-validation (RMSECV), in which accumulation of new LV did not actually enhance model performance, with a maximum of 8 LVs.

4. EXPERIMENTATION AND RESULT

4.1 Egg Freshness Evaluation:

The physicochemical changes occur in the egg immediately after it has been laid and cause the degradation in freshness index [24]. Once the egg is stored it's quality starts reducing due to loss of carbon dioxide and water through the egg shell. Gaseous exchange occurs during the storage period which increases the acidity and consistency of the albumen i.e. egg white by changes in ovomucin and lysozyme interaction during storage [11]. It affects the albumen height which was observed in HU value and helps in estimating the freshness of chicken egg.

Figure 1 illustrates the changes in HU values and egg freshness classes over the storage period at temperatures of 20°C and 30°C. In Fig. 1, a significant decrease in HU value over time is evident, with eggs stored at 30°C exhibiting a more pronounced decline compared to those at 20°C, particularly noticeable after 14 days. This suggests that eggs stored at 20°C can maintain freshness (HU > 60) for up to 14 days, while those stored at 30°C remain fresh for only 10 days. However, eggs maintain a notably elevated HU value when stored at 20°C even on day 21 (Fig.1). Consequently, storing eggs at higher temperatures, especially in tropical climates, Near-Infrared (NIR) spectra, unlike techniques such as Raman or Mid-Infrared (MIR) spectroscopy [13], are known for their lower information content, lacking distinct absorption peaks. Instead, NIR spectra exhibit high correlation among various wavelengths. Principal Component Analysis (PCA) proves to be exceptionally useful in handling such datasets efficiently. PCA is adept at reducing the dimensionality of multidimensional data, especially when strong correlations are present. By projecting the original multidimensional dataset (in this case, 601 dimensions) into a lower-dimensional space, often just a few or two dimensions, PCA eliminates redundancy and retains essential information, making it a powerful tool for simplifying and interpreting complex NIR spectral data [6].

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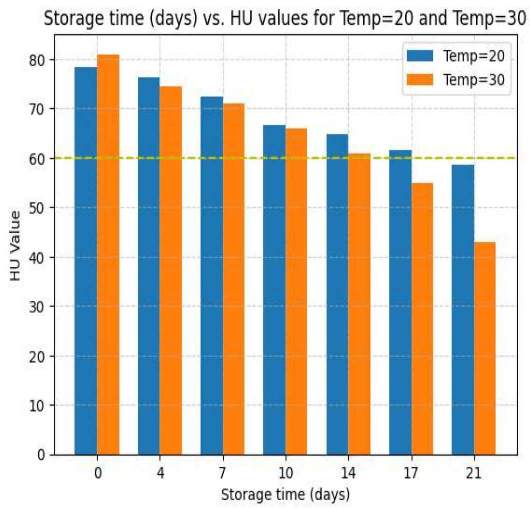

Figure 1: Storage Time vs HU (at 20°C and 30°C)

The PCA analysis conducted on the full spectra range of 902 to 1810 nm involved preprocessing using Savitzky-Golay (S-G) derivative after SNV transformation. After the removal of outliers, the initial two principal components (PC1 and PC2) were found to capture 98% of the data's variance. Given the direct association between egg grading and the HU scale, this specific spectral region presented an opportunity to develop enhanced precision in predictive models, enhancing information analysis efficiency. Subsequently, narrowing down the wavelength range considerably enhanced the grouping of egg categories, as demonstrated by the PCA scores for average spectra following SNV and 1st S-G derivative processing. PC1 captured nearly 98% of the data's variability. This reduction in the spectral region contributed to enhanced class separation and more effective analysis introduces greater variability in samples for regression models. This increased variation in response data may contribute to more accurate models, enhancing the reliability of RPD and RER measurements [8].

4.2 PCA Analysis:

Near-Infrared (NIR) spectra, unlike techniques such as Raman or Mid-Infrared (MIR) spectroscopy, are known for their lower information content, lacking distinct absorption peaks. Instead, NIR spectra exhibit high correlation among various wavelengths. Principal Component Analysis (PCA) proves to be exceptionally useful in handling such datasets efficiently. PCA is adept at reducing the dimensionality of multidimensional data, especially when strong correlations are present. By projecting the original multidimensional dataset (in this case, 601 dimensions) into a lower-dimensional space, often just a few or two dimensions, PCA eliminates redundancy and retains essential information, making it a powerful tool for simplifying and interpreting complex NIR spectral data [8].The PCA analysis conducted on the full spectra range of 902 to 1810 nm involved preprocessing using Savitzky-Golay (S-G) derivative after SNV transformation. After the removal of outliers, the initial two principal components (PC1 and PC2) were found to capture 98% of the data's variance. Given the direct association between egg grading and the HU scale, this specific spectral region presented an opportunity to develop enhanced precision in predictive models, enhancing information analysis efficiency. Subsequently, narrowing down the wavelength range considerably enhanced the grouping of egg categories, as demonstrated by the PCA scores for average spectra following SNV and 1st S-G derivative processing. PC1 captured nearly 98% of the data's variability. This reduction in the spectral region contributed to enhanced class separation and more effective analysis [7].

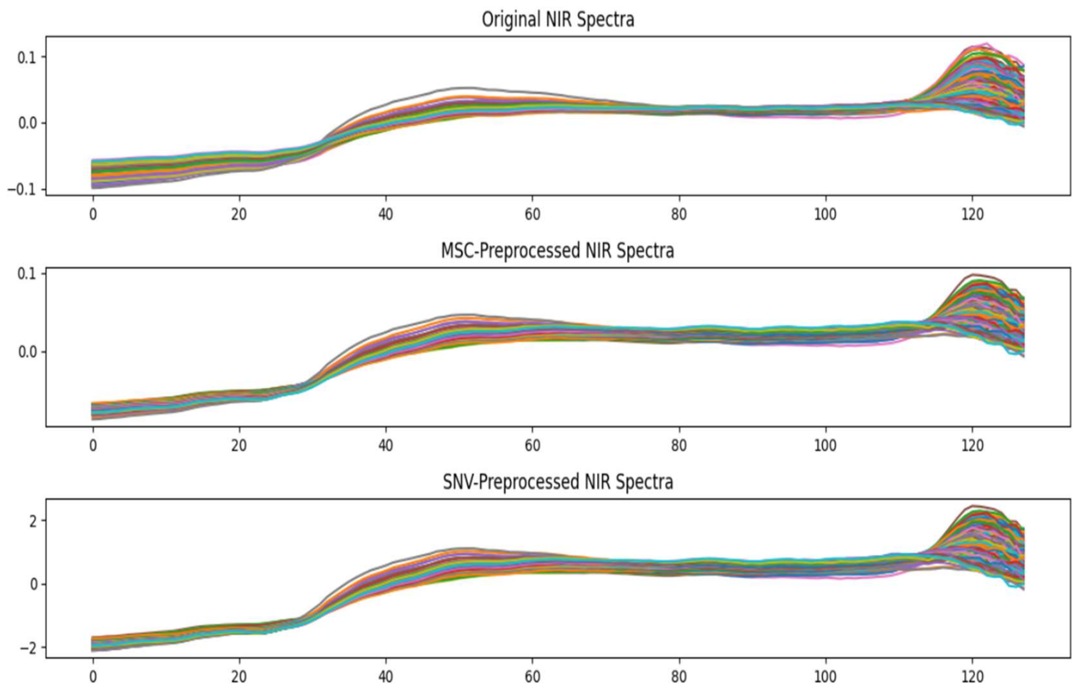

Figure 2: Preprocessed Spectra (MSC+SNV)

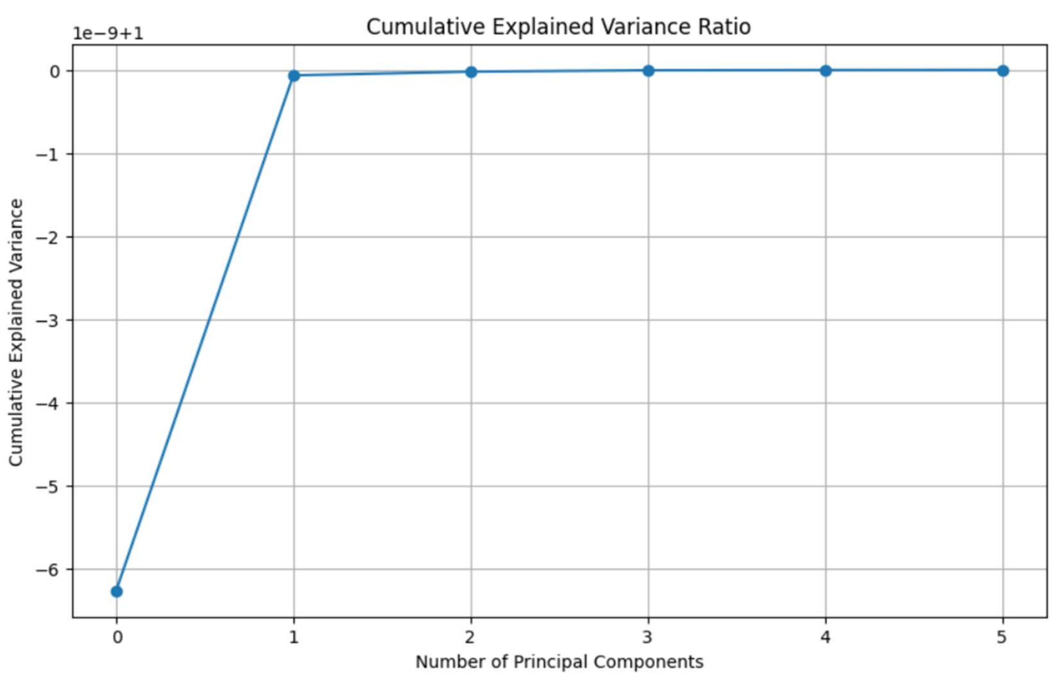

Figure 3: Principal Component Variance

4.3 Estimation of HU:

The performance characteristics of the PLS-Regression model, which considers several shell measurement areas, are shown in Table 1 for the purpose of predicting the HU value of eggs held at 20°C. Table 1 shows the effect of the different latent variables and shell measurement area on the R2 c (coefficient of determination for calibration), R2 cv (coefficient of determination for cross-

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validation), RMSEC (root mean square error for calibration), RMSECV (root mean square error for cross-validation), RPD (ratio of performance to deviation), and % Relative Error. The RPD greater than 2 is considered as the model is best suited for the prediction of target values. This model proved its efficiency and accuracy by providing low RMSE, high R2 c, highest RPD for the mean R1+R2+R3 shell area. The models are effective in predicting HU values for eggs at 20°C. These outcomes indicate that the PLS-R model's capacity to predict HU values is highly inclined by the amalgamation of certain preprocessing techniques, shell measurement areas with potential associations in estimating egg quality [8].

***Table 1** Evaluation Metrics for PLS-R Model Predicting HU in Eggs at 20°C*

Shell Measurement Area	LV	Preprocessing	Spectral Range	R^2_c	R^2_{cv}	RMSEC	RMSECV	RPD	% Relative Error
R1	8	SNV+SG+1st Derivate	902-1810 nm	0.984	0.963	1.282	1.953	5.165	3.091
R2	6	SNV+SG+1st Derivate	902-1810 nm	0.979	0.961	1.455	1.991	5.066	3.152
R3	7	SNV+SG+1st Derivate	902-1810 nm	0.982	0.964	1.370	1.919	5.258	3.037
Mean R1+R2	8	SNV+SG+1st Derivate	902-1810 nm	0.985	0.969	1.223	1.788	5.641	2.830
Mean R1+R3	7	SNV+SG+1st Derivate	902-1810 nm	0.985	0.967	1.256	1.835	5.438	2.904
Mean R1+R2+R3	8	SNV+SG+1st Derivate	902-1810 nm	0.986	0.970	1.196	1.756	5.743	2.780
Mean R2+R3	7	SNV+SG+1st Derivate	902-1810 nm	0.983	0.968	1.310	1.795	5.621	2.841

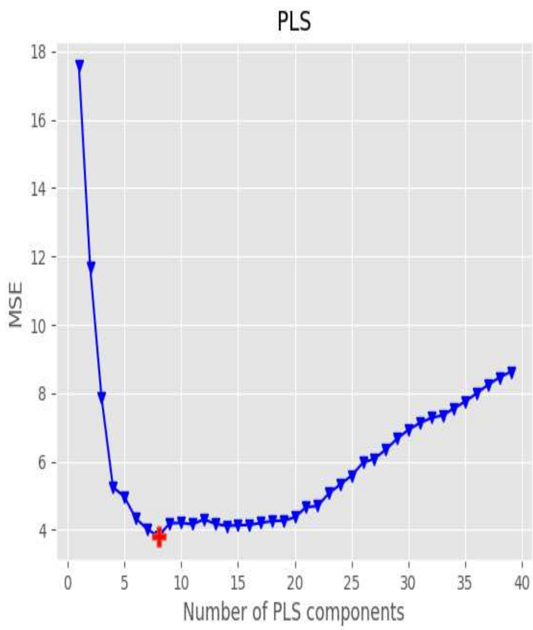

Figure 4: Number of PLS component (LV) for minimum MSE

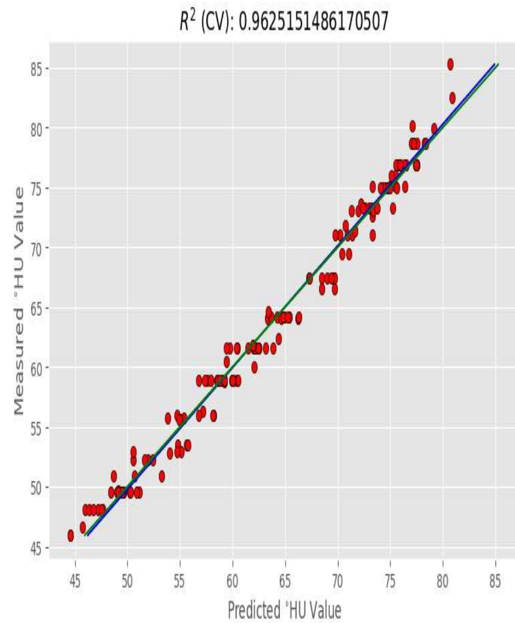

Figure 5: Predicted HU value vs Measured HU value graph.

4.4 Eggs discrimination using freshness index:

PLS-DA models with varying shell measurement regions, latent variables (LV), preprocessing methods, and spectral ranges were created for the freshness-based categorization of hen's eggs. Using 8 LV, Standard Normal Variate (SNV) transformation, Savitzky-Golay (SG) smoothing [26], and first derivative preprocessing spanning the 902–1810 nm spectral range, the model demonstrated a 93.75% accuracy rate in identifying the freshness classes in area R1. In a similar vein, regions R2 and R3, adopting the same preprocessing techniques and spectral range, and having 6 and 7 LV, respectively, showed accuracy of 90.86% and 93.06%. Combining measurement regions yielded even higher accuracy: 95.75%, 89.00%, 91.25%, and 95.7%, respectively, when Mean R1+R2, Mean R1+R3, Mean R2+R3, and Mean R1+R2+R3 were combined the efficaciousness of these models underscores the significance of pinpointing precise regions for measuring the shell and utilizing preprocessing methods to maximize precision in determining the freshness of eggs. The robustness of the constructed models was demonstrated by the effective discriminating between freshness classes made possible by the application of PLS-DA, a potent chemometric approach. The findings imply that certain shell regions plus appropriate preprocessing methods greatly enhance the PLS-DA models' accuracy and offer important new information for effective and non-destructive egg quality evaluation [25].

Table 2: *Parameters of the PLS-DA Model for Hen's Egg Classification Based on Shell Measurement Location:*

Shell Measurement Area	LV	Preprocessing	Spectral Range	Freshness Accuracy
R1	8	SNV+SG+1st Derivate	902-1810 nm	93.75%
R2	6	SNV+SG+1st Derivate	902-1810 nm	90.86%
R3	7	SNV+SG+1st Derivate	902-1810 nm	93.06%
Mean R1+R2	8	SNV+SG+1st Derivate	902-1810 nm	95.7%
Mean R1+R3	7	SNV+SG+1st Derivate	902-1810 nm	89.00%
Mean R1+R2+R3	8	SNV+SG+1st Derivate	902-1810 nm	95.75%
Mean R2+R3	7	SNV+SG+1st Derivate	902-1810 nm	91.25%

5. CONCLUSION

With an emphasis on predicting HU and storage time, this study has shown the potential of Near-Infrared (NIR) spectroscopy in conjunction with chemometric modeling as a formidable tool for the non-destructive assessment of egg quality. The study involved a methodical strategy to gathering data, preprocessing NIR spectra, and creating predicting models for these crucial characteristics of egg quality. In general, mean R1+R2+R3 location shows the highest accuracy for predicting HU. The proposed methodology can forecast the storage day and classify the chicken eggs by grading them with freshness accuracy over 95.75%.

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