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FT-NIR Spectroscopy and Explainable Machine Learning for Eggshell Based Classification of Organic and Conventional Market Eggs

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Abstract

The premium pricing of organic eggs creates incentives for product misrepresentation and motivates the development of rapid, non-destructive screening tools to differentiate labeled organic and conventional eggs. Fourier transform near-infrared (FT-NIR) spectroscopy coupled with machine learning was evaluated for classifying retail eggs marketed as organic versus conventional. A total of 147 eggs were measured and spectra were analyzed using partial least squares–discriminant analysis (PLS-DA), along with gradient-boosted tree models (XGBoost and LightGBM). The dataset was partitioned into training (70%) and testing (30%) sets. Among the evaluated models, PLS-DA achieved the best test performance with an overall accuracy of 91.0%. To improve interpretability, SHapley Additive exPlanations (SHAP) were used to identify wavelengths that most strongly contributed to model predictions. Influential regions included bands near ~1400 nm, consistent with O-H absorptions commonly associated with moisture-related spectral features, and regions near ~2300 nm, which are often associated with crystalline calcium-carbonate-related absorptions in the near-infrared and may reflect variation in mineral-associated spectral response of eggshell. These results indicate that FT-NIR spectroscopy combined with machine learning can differentiate retail eggs labeled as organic versus conventional under the conditions of this study, supporting further development of rapid screening approaches for market classification and quality assurance applications.

Keywords: Market eggs, organic eggs, optical sensing, fraud detection, egg industry.

1.Introduction

The premium market value of organic eggs has heightened the need for rapid, reliable, and non-destructive methods to authenticate production systems. Concerns regarding any mislabeling and fraudulent substitution in egg markets may necessitate objective analytical tools capable of distinguishing organically produced eggs from conventional ones. Hen diet and rearing conditions are well known to influence egg composition, including lipid, protein, and micronutrient profiles. These effects are not limited to the yolk and albumen but may also extend to the eggshell, which is formed through tightly regulated physiological processes and reflects the mineral metabolism of the hen.

The eggshell is a complex biomineralized structure produced in the shell gland (uterus) and represents a significant metabolic investment (Hasan and Khaliduzzaman, 2022; Khaliduzzaman et al., 2022). It performs essential biological functions, including mechanical protection, prevention of microbial invasion, regulation of gas and moisture exchange, and provision of calcium for embryonic skeletal development during incubation (A.R and Rahn, 1985; Khaliduzzaman et al., 2020). Structurally, the eggshell consists predominantly (approximately 95–97%) of calcium carbonate in the calcite form, embedded within a minor but functionally critical organic matrix composed of proteins, glycoproteins, and polysaccharides that regulate crystal nucleation and growth.

The eggshell exhibits a hierarchical microstructure comprising the mammillary layer, palisade layer, and outer vertical crystal layer, often covered by a cuticle. Variations in crystal morphology, porosity, and organic matrix composition may arise due to differences in nutrition, housing systems, and management practices. In particular, feeding strategies associated with organic production systems, such as access to pasture and diverse feed resources, may influence mineral utilization and shell formation, potentially leading to detectable differences in eggshell properties.

These characteristics make the eggshell a promising target for non-destructive analytical techniques. Near-infrared (NIR) spectroscopy has gained attention due to its rapidity and suitability for industrial applications. However, in highly scattering materials such as eggshells, spectral responses are influenced

not only by chemical composition but also by microstructural features. Light interaction within the shell involves multiple scattering and interference phenomena governed by parameters such as thickness, refractive index, and crystal organization (Khaliduzzaman et al., 2021).

Fourier-transform near-infrared (FT-NIR) spectroscopy offers advantages over conventional dispersive NIR systems, including improved signal-to-noise ratio and enhanced sensitivity to subtle spectral variations. In diffuse reflectance mode, incident radiation penetrates the eggshell and undergoes multiple scattering events, generating spectra that encode both compositional and structural information. This makes FT-NIR particularly suitable for capturing subtle differences associated with production systems.

The complexity of spectral data necessitates advanced analytical approaches for effective interpretation. Machine learning techniques, including linear methods such as partial least squares discriminant analysis (PLS-DA) and non-linear algorithms such as LightGBM and XGBoost, have demonstrated strong performance in food classification tasks. However, many of these models operate as black boxes, limiting their interpretability and practical acceptance in regulatory and industrial settings. In this context, explainable artificial intelligence (XAI) provides an important advancement by enabling interpretation of model predictions and identification of the most informative spectral features. XAI techniques can reveal the contribution of specific wavelength regions associated with chemical bonds or structural characteristics, thereby improving transparency, model validation, and scientific understanding (Ahmed et al., 2025). This interpretability is particularly valuable for ensuring trust, regulatory compliance, and broader adoption of machine learning-based authentication systems in the food industry.

Therefore, the objective of this study is to develop a non-destructive framework for the classification of organic and conventional market eggs based on eggshell properties using FT-NIR spectroscopy integrated with machine learning and explainable AI approaches. The proposed methodology has the potential to support rapid, reliable, and interpretable egg authentication in industrial grading systems, thereby enhancing consumer confidence and protecting the integrity of premium egg markets.

2.Materials and Methods

2.1 Materials

We followed the animal experiment protocol approved by the Office of the Vice-Chancellor for Research IACUC online protocols, University of Illinois (protocol #: 22224). A total of 147 eggs (both white and brown shell eggs) were measured (conventional = 87; organic = 60), and spectra were analyzed for prediction model development using partial least squares–discriminant analysis (PLS-DA) with common spectral preprocessing, along with gradient-boosted tree models (XGBoost and LightGBM). The dataset was partitioned into training (70%) and testing (30%) sets. To improve interpretability, SHapley Additive exPlanations (SHAP) were used to identify wavelengths that most strongly contributed to model predictions.

2.2 Spectra acquisition

Fourier-transform near-infrared (FT-NIR) spectroscopy (Model: TANGO-R, Bruker Corporation) was used to acquire egg spectra in the wavelength range of 866–2535 nm (Fig. 1). The TANGO FT-NIR spectrometer was equipped with an internal gold-coated integrating sphere for diffuse reflectance measurements of egg samples. The system operated using TANGO and OPUS software for spectral acquisition and data handling. Background measurements were performed automatically by the instrument without user intervention, and the system was not affected by ambient light conditions. The diameter of the measurement spot was approximately 10 mm. The interferometer, based on Cube Corner technology and highly reflective gold-coated optics, ensured optimized light throughput and spectral quality.

Unlike conventional dispersive near-infrared spectroscopy, FT-NIR spectroscopy utilizes an interferometer, which improves spectral resolution and signal-to-noise ratio. For eggshell measurements, the scan time and spectral resolution were set at 40 s and 8.0 cm^{-1}, respectively. Although spectra were initially acquired in reflectance (R) mode, the OPUS software automatically converted and stored them as absorbance values (log (1/R)).

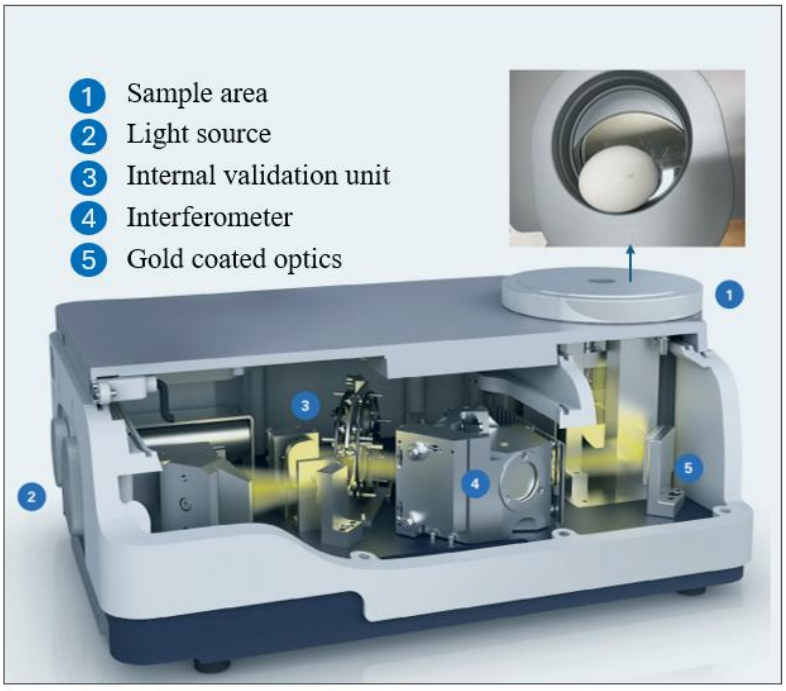


Figure 1: Schematic diagram of spectra acquisition including the internal components of the TANGO-R FT-NIR Spectroscopy.

The FTIR spectrometer used the following equations (eq.1-2) for calculating diffuse reflectance (R) and absorbance (A):

$$R = \frac{I_{egg}}{I_{background}} \quad \ldots \quad \ldots \quad (1)$$

$$A = \log (1/R) \quad \ldots \quad \ldots \quad (2)$$

where, I_{egg} is the intensity of infrared light diffusely reflected from the eggshell, and $I_{background}$ is the intensity of the light reflected from the background without sample.

2.3 Classification Models

Both linear and non-linear models were applied to the spectral data for the classification of conventional and organic eggs. A linear approach, partial least squares discriminant analysis (PLS-DA), and two non-linear models, Light Gradient Boosting Machine (LightGBM) and Extreme Gradient Boosting (XGBoost), were evaluated.

PLS-DA is a supervised linear classification method derived from partial least squares (PLS) regression. It reduces high-dimensional predictor variables, such as spectral data, into a set of latent variables by maximizing the covariance between the predictor matrix (X) and the response variable (Y). The response variable is encoded in a binary or dummy format, allowing class separation based on linear decision boundaries in the latent space. PLS-DA is particularly suitable for handling collinear and high-dimensional datasets, which are common in spectroscopic applications (Khaliduzzaman et al., 2025). LightGBM is a non-linear, tree-based ensemble learning algorithm based on gradient boosting. It constructs decision trees sequentially, where each new tree is trained to minimize the residual errors of the previous model using gradient-based optimization. Similarly, XGBoost is an advanced gradient boosting framework that builds an ensemble of decision trees in a stage-wise manner. It incorporates regularization techniques (L1 and L2), learning rate control, and feature subsampling to reduce overfitting and improve model generalization. XGBoost follows a level-wise tree growth strategy and supports parallel computation, making it efficient and scalable for large datasets (Sahin, 2020).

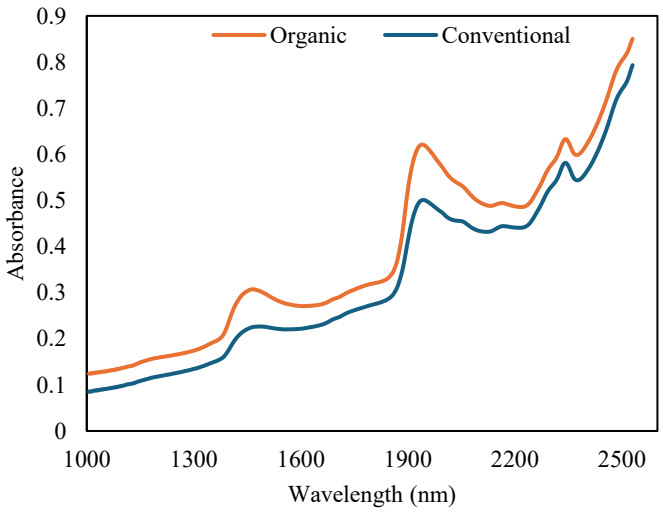
Model performance was evaluated using precision, recall, F1-score, and overall accuracy for both linear and non-linear approaches. Important variables contributing to the non-linear models were identified using explainable artificial intelligence techniques, specifically SHapley Additive exPlanations (SHAP), to improve model interpretability and identify influential spectral regions (Stenwig et al., 2022).

3. Results and Discussion

3.1 Typical spectra of intact eggshell

The average FT-NIR absorbance spectra of intact eggshells (Fig. 2) showed a consistent difference between organic and conventional eggs across the measured wavelength range (1000–2500 nm). In general, organic eggs exhibited higher absorbance than conventional eggs throughout the spectrum, suggesting differences in shell composition and/or structure. The major component of eggshells and palisade layer is crystalline $CaCO_3$ which acts as a shield for internal contents of an egg. The eggshell consisted of outer vertical crystal layers, long palisade layers and mammillary cones. The palisade layer is made of calcite crystals in which

171 an organic matrix is embedded, which accounts for the thickness of the eggshell (Park and Sohn, 2018). It
172 is clearly visible that two big shooting (peaks near 1450, 1950 nm) in NIR spectra as shown in fig. 2. These
173 two peaks might be influenced by the absorbance of embedded protein in palisade layer, mammillary cones,
174 membrane and/or thin albumen. Water also gives distinct peak at 1450 nm (Manley, 2014). Thus, it is
175 influenced by the moisture content of the material as well. And about 89.0 % content of thin albumen is
176 water (Hrabia, 2022). Therefore, the deeper the peak of NIR spectra may indicate higher absorption and
177 thus may indicate higher amount of organic matter in the metal-organic or inorganic-organic matrix of the
178 shell layers.



179

180 Figure 2. Average absorbance spectra of organic and conventional eggs of intact eggshells obtained from
181 the FT-NIR spectroscopy (1000-2500 nm).

182 A gradual increase in absorbance was observed from 1000 to around 1800 nm for both egg types, followed
183 by more pronounced spectral features at higher wavelengths. Two major absorbance peaks were clearly
184 visible near 1450 nm and 1950 nm. These bands were commonly associated with O–H stretching vibrations,
185 primarily related to water content, but may also include contributions from organic components such as
186 proteins present in the eggshell matrix, shell membranes, and residual albumen. The higher intensity of
187 these peaks in organic eggs may indicate relatively greater moisture retention or higher organic matrix
188 content within the shell structure.

Beyond 2000 nm, additional smaller spectral features appeared in the region of 2000-2400 nm. These bands are attributed to overtones and combination vibrations of carbonate (CO_3^{2-}) ions in calcium carbonate, the primary mineral component of the eggshell (Cao et al., 2024). Although these peaks are less intense than the major water-related bands, they can provide useful information about the crystallinity and microstructural organization of calcite. Slight differences in this region between organic and conventional eggs may reflect variations in crystal size, orientation, or the presence of minor impurities.

The overall higher absorbance observed in organic eggs, particularly around the major peaks and in the higher wavelength region, suggested differences in the inorganic-organic matrix of the shell. These differences may arise from variations in hen diet, mineral metabolism, and rearing conditions, which are known to influence eggshell formation.

In addition to chemical composition, the layered microstructure of the eggshell-comprising the mammillary layer, palisade layer, and outer crystal layer-affects light scattering during FT-NIR measurement. Multiple scattering events within these layers may contribute to the broad and smooth spectral features observed, rather than sharp peaks. Therefore, the spectra reflected a combined effect of both chemical constituents and physical structure of the eggshell.

3.2 Principal Component Analysis

Principal component analysis (PCA) is an unsupervised multivariate technique used to reduce data dimensionality while preserving most of the variation present in the dataset. It is commonly applied to spectral data to explore patterns, detect variability, and assess the feasibility of classification before applying supervised models (Islam and Khaliduzzaman, 2022). PCA does not use class labels; instead, it transforms the original variables into a new set of orthogonal components (principal components) based only on the input features. As shown in Fig. 3, the PCA score plot indicates a visible separation between conventional and organic eggs based on their spectral characteristics. This separation suggests that the two groups differ in their absorbance profiles, which is consistent with the differences observed in the FT-NIR spectra. The first principal component (PC-1) explains 94.76% of the total variance, indicating that most of

the variability in the data is captured along a single direction. In contrast, PC-2 explains only a small proportion (3.98%) of the variance and contributes less to class discrimination.

Although some overlap between the two classes is still present, the overall trend showed that organic samples are more concentrated in one region, while conventional samples are more dispersed along the PC-1 axis. This pattern suggested that the primary differences between the two egg types are largely one-dimensional and may be related to systematic variations in eggshell composition or structure.

The observed separation in PCA is an important preliminary indication that the spectral data contain meaningful information for classification. However, since PCA is an unsupervised method and does not maximize class separation, it cannot fully distinguish between the two groups. Therefore, more advanced supervised machine learning models are required to improve classification accuracy. The combination of PCA with supervised learning and explainable AI approaches can enhance both prediction performance and interpretability. For example, PCA can be used for noise reduction and feature compression before model training, while explainable methods such as SHAP can help identify the most important spectral regions contributing to class separation. This integrated approach can support the development of robust and interpretable models for practical egg authentication.

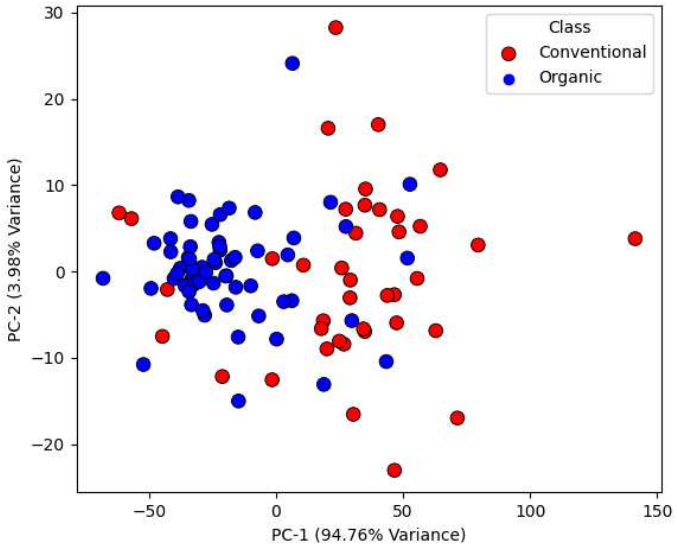


Figure 3. Principal component analysis of the eggs' spectra. Moderate separation is clearly visible in PC score plot where PC-1 explained most of the variance.

3.3 Models Performances

The performance of the linear and non-linear classifiers (PLS-DA, LightGBM, and XGBoost) is summarized in Table 1 using precision, recall, F1-score, and overall accuracy. Among the tested models, PLS-DA achieved the highest performance, with a precision of 0.92, recall of 0.91, F1-score of 0.91, and an overall accuracy of 91.0%. This indicates a well-balanced model with low misclassification rates. XGBoost showed the second-best performance, with precision, recall, and F1-score values of 0.90, 0.89, and 0.89, respectively, and an accuracy of 89.0%. LightGBM showed comparatively lower performance, with an overall accuracy of 84.0%.

These results are consistent with the PCA findings (Fig. 3), where a moderate separation between organic and conventional eggs was observed mainly along the PC-1 axis, which explained more than 94% of the total variance. The strong performance of PLS-DA suggests that the class differences are largely linear and can be effectively captured in a reduced latent variable space. This aligns with the PCA result, where most of the variation and thus class-related information was concentrated along a single principal component.

Although non-linear models such as XGBoost also performed well, their advantage over the linear model was limited. This further supports the interpretation that the underlying differences between organic and conventional eggshell spectra are not highly complex or non-linear. Instead, they appear to follow a relatively simple pattern, likely driven by systematic variations in eggshell composition and structure. The slightly lower performance of LightGBM may be due to its sensitivity to parameter tuning and the relatively small dataset.

Table 1. Performance of prediction models to classify conventional and organic eggs

Model	Precision	Recall	F1-Score	Overall Accuracy (%)
PLS-DA	0.92	0.91	0.91	91.0
LightGBM	0.87	0.84	0.85	84.0
XGBoast	0.90	0.89	0.89	89.0

The classification models were developed using a combined dataset of brown and white eggshells, which may introduce additional variability due to inherent physicochemical differences between shell types. This variability can increase within-class variation and reduce model performance. Conducting separate analyses for different shell colors may help improve classification accuracy. In addition, eggs categorized as organic were obtained from free-range systems, where hens may have variable access to pasture, insects, and supplemental feed. Such variation in diet and environmental exposure can introduce heterogeneity within the organic class. Future studies should consider more controlled definitions of production systems or include detailed information on feeding practices and environmental conditions. This could reduce variability and improve model robustness.

3.4 Important variables selection

Explainable artificial intelligence was applied to identify the most important variables contributing to the classification models. In this research, SHapley Additive exPlanations (SHAP) were used to quantify the contribution of each wavelength to model predictions. SHAP is based on game theory and assigns an importance value to each feature by fairly distributing its contribution across all input variables. This approach improves the interpretability of complex machine learning models and helps relate spectral features to underlying biological processes. A bee swarm plot (Fig. 4) illustrated the distribution of SHAP values across all samples. The most influential wavelengths are shown at the top of the plot. Each point represented an individual observation, where red indicated higher feature values and blue indicates lower values. In binary classification, SHAP values indicate how each wavelength contributes to shifting the prediction toward either organic or conventional eggs. Features at the top indicated the most influential wavelengths and red higher feature values whereas blue indicated lower value. In binary classification, SHAP breaks down the prediction for the positive and negative class. For each individual instance, SHAP values show how features push the prediction towards one class (positive) or the other (negative).

The most important wavelengths identified for the non-linear models are summarized in Table 2. The selected wavelengths were mainly grouped into three spectral regions: around 1000 nm, 1400 nm, and 2300 nm. Each of these regions can be linked to specific physicochemical and biological characteristics of the eggshell.

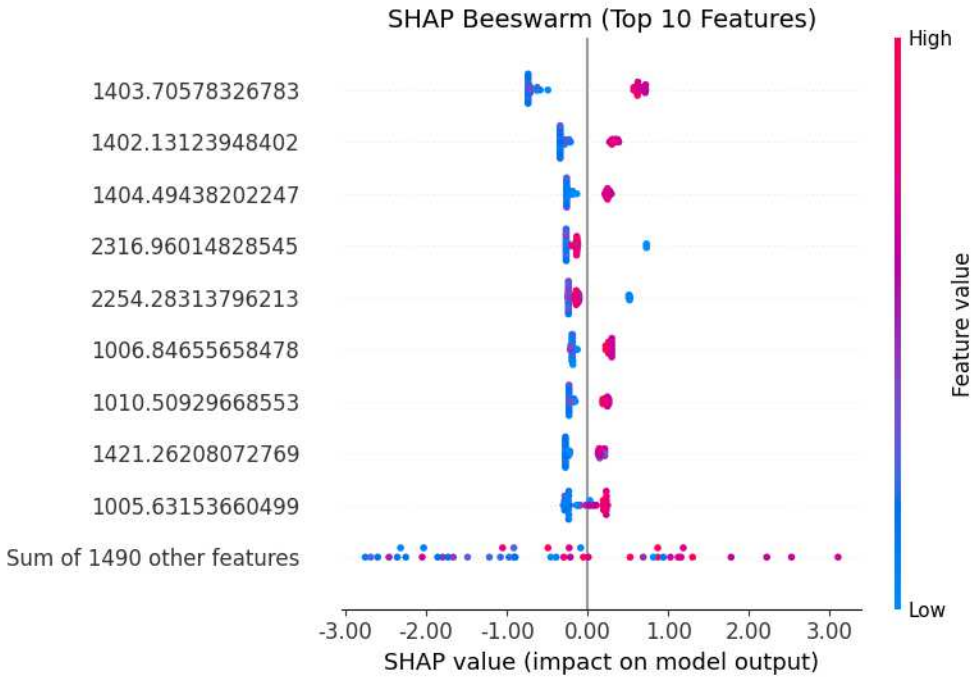


Figure 4. Bee swarm plot explaining important variables contributed to XGBoast model based on SHAP value.

The band near 1400 nm (e.g., 1403 nm) is strongly associated with O–H stretching vibrations, which are primarily related to water content (Caner and Yüceer, 2015). This includes moisture present in the eggshell, shell membranes, and at the shell-albumen interface. Differences in this region may reflect variations in water retention and hydration of the organic matrix. These properties are influenced by hen physiology and nutrition, particularly protein intake and metabolic activity during shell formation. The spectral region around 2300 nm is associated with carbonate (CO_3^{2-}) overtones and combination bands, which are directly related to calcium carbonate crystallinity. Since the eggshell is predominantly composed of calcite, this region provides information about mineral structure, crystal size, and degree of organization. Variations in this band suggest differences in mineral deposition and shell formation processes. These differences can be

influenced by calcium availability, dietary mineral balance, and vitamin D metabolism, which regulate calcium absorption and transport in laying hens. The region near 1000 nm is linked to the organic matrix of the eggshell, including proteins, glycoproteins, and polysaccharides embedded within the palisade layer and mammillary cones. This organic matrix might play a critical role in crystal nucleation and growth during biomineralization. Variations in this region may indicate differences in the composition or distribution of organic components, which are influenced by diet quality, amino acid supply, and overall hen health.

Table 2. Important variables based on SHAP values contributed to non-linear models

Model	Method	Important variables
LightGBM	SHAP-Bee Swarm	1403, 1006, 1412, 1010, 1021
XGBoast	SHAP-Bee Swarm	1403, 2316, 2254, 1421, 1010

From a biological perspective, hens in organic or free-range systems often have more variable diets, including access to pasture, insects, and supplemental feed. This can affect both mineral metabolism and organic matrix formation, leading to subtle but measurable differences in eggshell composition and structure. In contrast, conventional systems typically provide more controlled and uniform diets, which may result in more consistent mineral deposition but potentially lower variability in organic components.

Therefore, the SHAP analysis demonstrated that the classification of organic and conventional eggs is driven by a combination of moisture-related features, mineral crystallinity, and organic matrix composition. These findings are consistent with the FT-NIR spectral patterns and PCA results, confirming that both chemical and structural differences in the eggshell contribute to model predictions. Importantly, the use of explainable AI thus provided a clear link between spectral data and biological mechanisms, improving the transparency and scientific relevance of the classification models.

4. Conclusions

This research focused the potential of FT-NIR spectroscopy combined with machine learning and explainable artificial intelligence for the classification of organic and conventional market eggs based on eggshell characteristics. The FT-NIR spectra revealed consistent differences between the two egg types, particularly in regions associated with water, organic matrix components, and calcium carbonate structure. Organic eggs generally showed higher absorbance, suggesting subtle variations in moisture content and eggshell composition. Unsupervised PCA analysis indicated a clear but moderate separation between organic and conventional eggs, with most variability explained by a single principal component. This suggested that the primary spectral differences are relatively systematic and largely captured in a reduced dimensional space. Based on this observation, supervised classification models were effectively developed. Among the tested models, partial least squares discriminant analysis (PLS-DA) achieved the best overall performance, followed closely by XGBoost, while LightGBM showed comparatively lower accuracy. The strong performance of the linear model indicated that the spectral differences between organic and conventional eggs are largely linear in nature and can be effectively captured using latent variable-based approaches. Explainable AI using SHapley Additive exPlanations (SHAP) further identified the most influential spectral regions contributing to classification. Key wavelengths were primarily located around 1000 nm, 1400 nm, and 2300 nm, which are associated with organic matrix components, water-related vibrations, and calcium carbonate crystallinity, respectively. These findings provided important biological insights, linking spectral variation to eggshell formation processes influenced by hen diet, mineral metabolism, and rearing conditions. The results confirmed that eggshell spectral signatures contain meaningful chemical and structural information that can be effectively used for classification. Future studies could focus on expanding sample diversity, standardizing organic production definitions, and integrating additional spectroscopic modalities to further improve model robustness and generalization for industrial applications-rapid screening approaches for market classification and quality assurance applications.

Funding Declaration

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Ethics Declaration

Although research manuscripts didn't use any data of any live animal, this experiment was conducted according to the animal experiment protocol approved by the Office of the Vice-Chancellor for Research IACUC on-line protocols, University of Illinois (protocol no. 22224).

Competing interests

Alin Khaliduzzaman is on the editorial board member of this journal.

Author contributions

Alin Khaliduzzaman: Conceptualization, Methodology, Investigation, Data curation, Formal analysis, Writing – original draft. Mohammed Kamruzzaman: Sample-collection, research funding. Isabella Condotta: Writing – review and editing, Project administration.

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