

Effects of Simulated Degradation Conditions on the Authentication of Fresh Quail Eggs Using a Miniaturized NIR Spectrometer and DD-SIMCA

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This study developed a reliable and non-destructive analytical method for quality control of quail eggs under simulated degradation conditions, including temperature variations and UV radiation exposure, using a miniaturized near-infrared (NIR) spectrometer combined with Data-Driven Soft Independent Modeling of Class Analogy (DD-SIMCA). A total of 336 quail egg samples were evaluated, with 90 samples stored under optimal conditions for up to 14 days, while the remaining 246 samples underwent controlled storage at temperatures of 30 and 40 °C for 24, 48, and 72 h, and were exposed to UV irradiation for 2, 2.5, 3, 3.5, and 4 h. DD-SIMCA models utilizing NIR spectra pre-processed with multiplicative scatter correction (MSC) and Savitzky-Golay first derivative (SGD) achieved remarkable sensitivity and specificity of 100% in the test set, demonstrating an effective and low-cost technology for ensuring the authenticity of fresh quail eggs.

Keywords: *Coturnix coturnix japonica*, food storage, quality control, vibrational spectroscopy, pattern recognition, one class classification

Introduction

The Japanese quail (*Coturnix coturnix japonica*), a domesticated avian species originating from East Asia, is predominantly reared in Asia and Europe for both recreational purposes and the production of meat and eggs.¹ This species is a favored animal model in biological and genetic research due to its noteworthy disease resistance, rapid growth rate, early sexual maturity (from 39 to 50 days), ease of management, and the feasibility of maintaining large populations within confined spaces.²⁻⁴ Interest in Japanese quail husbandry is notably prevalent among small-scale breeders, attributable to the species' high reproductive efficiency and brief incubation period of approximately 17 days.⁵ In such a scenario, the commercial

breeding of Japanese quail has emerged as a significant and expanding industry in Brazil. Data from the Brazilian Institute of Geography and Statistics (IBGE, Brazil) estimate that quail egg production in Brazil reached 2.75 billion units, generating a production value of 431.537 million reais in 2022.⁶

Given the egg consumption provides an affordable source of essential nutrients, further research on egg composition and technological quality is warranted.⁷ Indeed, many avian species exhibit similarities in nutritional composition and potential for food use.⁸ The modern egg industry traditionally mandates specific criteria for egg integrity to ensure proper commercialization. These criteria include eggshell integrity and color, both of which significantly impact consumer visual perception and, consequently, market acceptance.⁹ However, assessing egg quality is a more complex task, involving parameters like size, weight, color, defects, spoilage, bacterial infection, and freshness. Freshness, a key quality criterion, depends on biochemical and physical

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attributes. Aging impacts egg quality immediately post-oviposition, causing water and CO₂ loss through shell pores, reducing weight, increasing pH, and liquefying albumen and yolk. Environmental conditions, especially temperature, humidity, and light exposure, further affect egg quality during storage,¹⁰⁻¹⁴ and have been associated with alterations in internal quality characteristics. Therefore, it is crucial to maintain optimal internal and external quality during storage and incubation.^{15,16}

There is limited research available on the quality characteristics of quail eggs, including destructive^{17,18} and non-destructive^{14,19-21} analysis. Kumbár *et al.*¹⁷ evaluated the effect of storage duration on the egg quality parameters (egg weight, shell weight, yolk height, albumen height, albumen index, yolk index, Haugh units, and egg weight loss) and on the rheological characteristics of the liquid egg products. Quail eggs were stored for 16 weeks at 4 °C while maintaining acceptable internal quality parameters. Liquid egg products displayed Non-Newtonian fluid behavior, with their rheological characteristics accurately described by both the Herschel-Bulkley and Ostwald-de Waele models, indicating their complex flow dynamics under varying shear conditions. Apparent viscosity decreased with storage time, with egg liquids significantly affecting the eggshell surface displacement response. Castro *et al.*¹⁸ evaluated shell thickness, Haugh unit, and internal quality unit under different air temperatures and subsequently established thermal comfort thresholds for Japanese quails in terms of the egg quality. They found that the optimal intervals were 68.4 to 76.2 for the temperature-humidity index, 69.1 to 77.2 for the black globe humidity index, and 50.5 to 67.2 kJ kg⁻¹ of dry air for enthalpy.

Among the non-destructive methods, Nakaguchi and Ahamed¹⁹ employed a thermal microcamera for detecting embryos and assessing freshness (in terms of the air cell enlargement)¹⁴ in quail eggs via thermal images during different incubation periods. Deep learning object detection algorithms distinguished between fertilized and unfertilized eggs during the first 168 h (7 days) of incubation, with average precisions between 91.8 and 99.5% in the training models, but results were considerably reduced in the test sets.¹⁹ On the other hand, the lack of freshness presented a negative correlation ($R^2 = 0.676$) between air cell area and weight loss considering periods of 30, 50, and 60 days after the labeled expiration date. Deep learning models achieved F1 scores between 0.69 and 0.89.¹⁴

Most recently, portable near-infrared (NIR) spectrometers coupled with machine learning modeling have also been employed. Lanza *et al.*²⁰ evaluated the capability of (portable) Vis/NIR and (benchtop and portable) NIR instruments to discriminate among table eggs

from quails fed with varying inclusion levels of silkworm pupa meal (SWM). Four experimental groups (0, 4, 8, and 12% SWM) were used, with classification models developed using partial least squares-discriminant analysis (PLS-DA), K-nearest neighbor (KNN), and support vector machine (SVM). NIR devices showed good accuracy (> 90%), particularly in the 1350-1600 and 1850-2200 nm spectral regions, demonstrating the efficacy of portable NIR spectrometers in at-line monitoring eggs from SWM-fed quails. Moreover, Brasil *et al.*²¹ estimated quail egg freshness, comparing Haugh unit (HU), yolk index (YI), and egg quality index (EQI) as reference methods. Using PLS and SVM regression for prediction and PLS-DA and SVM for classification, the study found EQI to be the best freshness indicator, with SVM regression achieving a ratio of performance to deviation (RPD) = 2.0-2.5 and range error ratio (RER) >10, while PLS-DA and SVM classification models discriminated over 80% of samples correctly.

Indeed, elevated temperatures above 30 °C detrimentally affect quail egg quality by reducing egg mass, specific gravity, Haugh unit, yolk index, and albumen height, with pronounced declines at 32 °C or higher.²²⁻²⁵ Heat stress and UV exposure also weaken immune responses, indirectly impairing egg quality.²⁵ Moreover, although controlled UV light exposure may enhance vitamin D synthesis and offer potential benefits, its adverse effects under prolonged or unregulated conditions often outweigh these advantages. UV light degrades the protective cuticle layer of quail eggs, which is essential for maintaining freshness by preventing microbial invasion and moisture loss. This degradation decreases protoporphyrin levels, lowering fluorescence intensity, a key freshness indicator.²⁶ While selective UV application during egg selection can improve hatchability, prolonged exposure compromises internal quality and reduces hatch rates due to structural damage.²⁷ These findings emphasize the importance of carefully managing environmental factors and UV exposure to ensure optimal egg quality.

So far, only the study by Brasil *et al.*²¹ has explored a portable NIR device for detecting quail egg freshness. Although the classification results were moderate, the study employed discriminant analysis rather than one-class classification (OCC), which is necessary for effectively addressing authentication issues.²⁸ In such a scenario, DD-SIMCA (Data-Driven Soft Independent Modeling of Class Analogy) ensures that samples from the target class are characterized independently of other classes, by defining the acceptance region using orthogonal and score distances exclusively from training samples. DD-SIMCA has already demonstrated robust applicability with portable NIR data from animal-origin food.²⁹⁻³¹

This study investigates the effect of simulated degradation conditions (temperature and UV radiation exposure) on the storage of fresh quail eggs, utilizing spectral fingerprints obtained via a miniaturized NIR spectrometer combined with DD-SIMCA. Ensuring the preservation of quail egg integrity and consumer safety under varying storage conditions is essential and requires sensitive, rapid, and cost-effective methodologies. The proposed approach facilitates real-time, *in situ* analysis across the supply chain, aligning with the principles of the Egg Industry 4.0 (EI 4.0). EI 4.0 leverages advanced technologies such as the Internet of Things (IoT), optical sensors, artificial intelligence (AI), and big data to improve automation, biosecurity, and quality inspection in egg production. Applying EI 4.0 principles to quail egg quality control enables the development of efficient, non-destructive, and sustainable analytical methods, addressing contemporary demands for precision and resource optimization in the egg industry.^{32,33}

Experimental

Samples

A total of 336 quail egg samples were examined in this study, stored for a duration of up to 14 days. Of these, 192 samples were kindly supplied by the aviary of the Animal Science Program at the Federal University of Paraíba (UFPB), Campus II, in Areia, Paraíba, Brazil. An additional 144 samples (representing six different brands) were purchased from supermarkets in João Pessoa, Paraíba, Brazil. The birds at the UFPB aviary were provided with feed and water *ad libitum*, whereas the commercial samples did not include detailed nutritional information on their labels.

To establish the target class, 90 fresh egg samples were selected. The training set included 36 samples from the UFPB aviary and 24 commercial samples (with 4 units from each of the six brands), totaling 60 samples. The target samples designated for the test set consisted of 6 samples from the UFPB aviary and 24 commercial samples (4 units from each of the six commercial brands), totaling 30 samples. The remaining 246 non-target samples (150 from the UFPB aviary and 96 commercial eggs) underwent controlled storage conditions at varying temperatures (30 and 40 °C) for periods of 24, 48, and 72 h, as well as exposure to UV irradiation for 2, 2.5, 3, 3.5, and 4 h. Additionally, various combinations of temperature, UV irradiation, and exposure time were applied as follows: UV irradiation for 4 h (10 samples); 40 °C for 24 h (10 samples); 40 °C for 48 h (10 samples);

UV irradiation for 2 h followed by 40 °C for 24 h (5 samples); and UV irradiation for 2 h combined with 40 °C for 24 h (5 samples).

Instrumentation

Temperature-controlled storage was conducted in an incubator (Q317M-43, Quimis®, Brazil) with a temperature control accuracy of ± 0.1 °C. UV radiation exposure was performed using a commercial UV light source (Fotolight-MD2-A4, Carimbos Medeiros Ltda, Brazil), equipped with two sets of mercury lamps (BLB-15 W-T8, SCT black light).

NIR spectra were recorded in triplicate from 900 to 1700 nm using a portable DLP NIRscan Nano Evaluation Module (Texas Instruments®) at 25 ± 1 °C. Diffuse reflectance was measured with a 2 nm pixel width and 228 points of digital resolution, using 32 scans with a Hadamard transform. A polytetrafluoroethylene powder was used as the blank. Only the average spectrum of each sample was used for chemometric models.

Chemometric procedures

Initially, a 7-point moving window smoothing was applied and, subsequently, six different pre-processing methods were employed to mitigate the effects of noise and baseline systematic variations: (i) offset correction (OFF), (ii) linear baseline correction (LBC), (iii) OFF + LBC, (iv) standard normal variate transformation (SNV), (v) multiplicative scatter correction (MSC), and (vi) Savitzky-Golay first derivative with a second-order polynomial and a 3-point window (SGD).

To perform an exploratory analysis of the data, principal component analysis (PCA) was employed. Subsequently, DD-SIMCA models were constructed using a significance level of 0.05 for the type I and II errors and for the significance of outliers. All samples under different simulated degradation conditions were treated as a single alternative class in the calculation of the type II error. The acceptance area was based on the chi-square distribution in the classic mode and the optimization of the number of principal components (PCs) employed the compliant approach. The performance of the DD-SIMCA models was evaluated in terms of sensitivity (calculated as the number of true positive (TP) decisions divided by the total number of positive cases), specificity (calculated as the number of true negative (TN) decisions divided by the total number of negative cases), and accuracy (calculated as the sum of all TP and TN divided by the total number of samples).³⁴

All chemometric procedures were performed using Matlab® 2018b (Natick, MA, USA, Mathworks).³⁵ PCA³⁶ and DD-SIMCA³⁷ were performed using proper Matlab toolboxes.^{38,39}

Results and Discussion

Preliminary considerations and exploratory analysis

Given the high similarity observed in the NIR spectra of the quail eggs under study, various pre-processing methods were employed. For illustration, Figure 1a presents the average NIR spectra of fresh (purple line) and non-fresh (gray line) quail egg samples, pre-processed with SGD. The application of the first derivative, while correcting for baseline shifts, enhances spectral resolution by increasing the number of distinguishable bands, thus improving the visualization and identification of differences between the samples. Figure 1b highlights the selected region from 1380 to 1540 nm, showing details of the average SGD spectra of non-fresh quail egg samples under controlled storage conditions: 30 °C for 24 h (yellow line), 40 °C for 24 h (orange line), 40 °C for 72 h (red line), UV for 24 h (green line), and UV for 4 h (blue line). The primary differences in this NIR region correspond to the first overtone of O–H stretching bonds, attributed to water loss through the shell and moisture content within the egg, as well as to N–H secondary bonds from amine groups, indicating potential protein structural changes during storage. Other pronounced features (Figure 1a) were observed between 1100 and 1250 nm, which are linked to C–H stretching, characteristic of saturated fatty acids in the yolk. Particularly, the prominent absorption band around 1150 nm corresponds to the second overtone of C–H absorption of pure fatty acids containing *cis* double bonds (C=O and C–H), likely reflecting higher oleic acid content in quail egg yolk.^{20,21} To illustrate, fresh quail eggs, when boiled, exhibit yolks with a distinct circular symmetry, centrally positioned within the albumen, and lacking an air chamber, as depicted in Figure 1c. In contrast, quail eggs subjected to simulated storage conditions display notable alterations in these structural features, which are reflected in their NIR spectra (Figure 1b). The differences in the NIR spectra are primarily attributable to changes in light scattering patterns, likely resulting from the formation of an air chamber and the degradation of the inner membrane, as shown in Figure 1d. These changes are closely linked to chemical and physical transformations during storage. Specifically, the albumen pH increases due to the loss of CO₂ through the eggshell, promoting hydrolysis of amino acid chains. This process releases water bound to large

proteins, increasing its permeability and allowing excess water transfer into the yolk.²¹ This water transfer flattens or distorts the yolk, disrupting its original symmetry and further altering the egg's light scattering properties as captured by the NIR spectra (Figure 1b).

To verify the feasibility of using NIR spectral fingerprints to differentiate fresh quail eggs from non-fresh quail eggs under simulated degradation conditions, PCA was initially explored to visualize natural trends of grouping between the studied samples. Figure 2 shows the average pre-processed spectra, with shaded areas representing the maximum variability observed at each wavelength, along with their corresponding PCA score plots obtained for OFF (Figures 2a and 2b), LBC (Figures 2c and 2d), OFF + LBC (Figures 2e and 2f), SNV (Figures 2g and 2h), MSC (Figures 2i and 2j), and SGD (Figures 2k and 2l), respectively. As observed, there is significant overlap between fresh (purple circles) and non-fresh (gray circles) quail egg samples across all pre-processing techniques, which necessitates the use of a supervised pattern recognition method to circumvent this issue.

Authentication of fresh quail eggs

Table 1 presents the confusion matrix for the best results obtained by DD-SIMCA for the authentication of fresh quail egg samples using NIR spectroscopy pre-processed with different techniques.

Since the DD-SIMCA models were constructed with a predefined α -value of 0.05 (i.e., considering *a priori* estimates of 95%), the *a posteriori* sensitivity obtained for all pre-processing techniques was higher than 95% and thus deemed adequate for predictive purposes. The purple and orange lines in Figure 3 indicate the thresholds established for the acceptance and outlier detection areas, respectively. Purple circles within the acceptance area represent regular fresh quail egg samples (purple circles) that were correctly projected, while orange squares located between the purple and orange lines are assigned as extremes. Additionally, no outliers were detected. As observed, three extreme samples were detected using OFF (Figure 3a), LBC (Figure 3b), MSC (Figure 3e), and SGD (Figure 3f), while two and four extremes were noted for OFF+LBC (Figure 3c) and SNV (Figure 3d), respectively. This indicates sensitivities of 97.8, 96.7, and 95.6% for models containing two, three, and four extremes, respectively, as shown in Table 1.

After delimiting the acceptance area for all pre-processing techniques studied, they were applied to verify the predictive ability of the constructed DD-SIMCA models in the projection of the test samples of both fresh

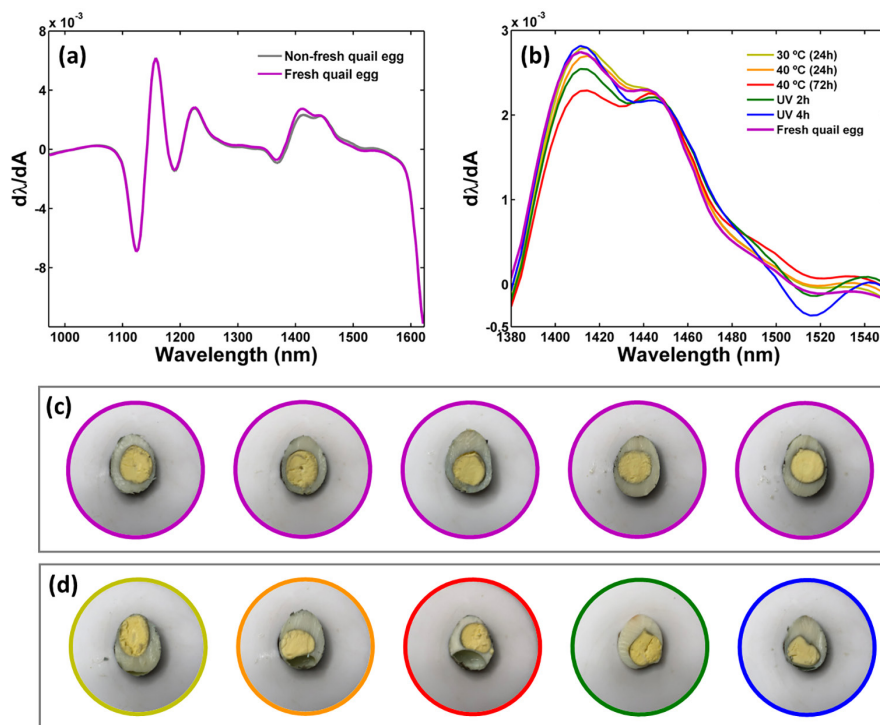


Figure 1. (a) Average NIR spectra of fresh (purple line) and non-fresh (gray line) quail egg samples, pre-processed with Savitzky-Golay first derivative with a second-order polynomial and a 3-point window. (b) Enlarged view of the selected region from 1380 to 1540 nm, showing details of the average derivative spectra, along with cross-sections of (c) fresh and (d) non-fresh boiled quail eggs under controlled storage conditions: fresh (purple line), 30 °C for 24 h (yellow line), 40 °C for 24 h (orange line), 40 °C for 72 h (red line), UV for 24 h (green line), and UV for 4 h (blue line). The images display changes in the internal and appearance of the eggs corresponding to different experimental conditions.

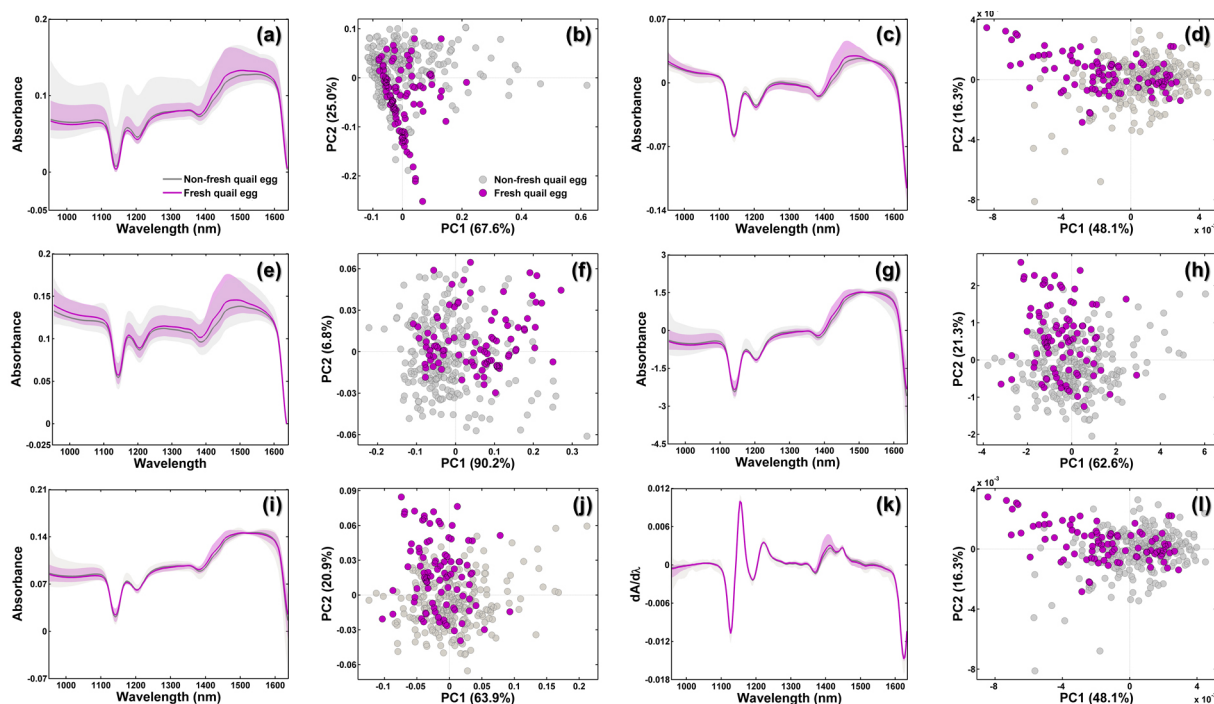


Figure 2. Average spectra, with shaded areas representing the maximum variability observed at each wavelength, along with their corresponding PCA score plots obtained for the NIR spectra of fresh (purple) and non-fresh (gray) quail egg samples, pre-processed with a 7-point moving window smoothing followed by (a, b) offset correction (OFF), (c, d) linear baseline correction (LBC), (e, f) OFF + LBC, (g, h) standard normal variate transformation (SNV), (i, j) multiplicative scatter correction (MSC), and (k, l) Savitzky-Golay first derivative with a second-order polynomial and a 3-point window (SGD), respectively.

Table 1. Best results (confusion matrix, with the sensitivity, specificity, and accuracy) obtained by DD-SIMCA ($\alpha = 0.05$) for the authentication of fresh quail eggs using miniaturized NIR spectroscopy

Pre-processing	Sample set	DD-SIMCA		
		PCs	Training	Test
			Target	Target Non-target
OFF	target (fresh quail egg)	9	87	29 1
	non-target		3	0 246
	Sensitivity / %		96.7	96.7
	specificity / %		–	100
	accuracy / %		98.8	
LBC	target (fresh quail egg)	7	87	30 0
	non-target		3	1 245
	sensitivity / %		96.7	100
	specificity / %		–	99.6
	accuracy / %		98.8	
OFF + LBC	target (fresh quail egg)	6	88	29 1
	non-target		2	0 246
	sensitivity / %		97.8	96.7
	specificity / %		–	100
	accuracy / %		99.1	
SNV	target (fresh quail egg)	7	86	29 1
	non-target		4	0 246
	sensitivity / %		95.6	96.7
	specificity / %		–	100
	accuracy / %		98.8	
MSC	target (fresh quail egg)	6	87	30 0
	non-target		3	0 246
	sensitivity / %		96.7	100
	specificity / %		–	100
	accuracy / %		99.1	
SGD	target (fresh quail egg)	7	87	30 0
	non-target		3	0 246
	sensitivity / %		96.7	100
	specificity / %		–	100
	accuracy / %		99.1	

Gray shade indicates the cell where the true positives occur. OFF: offset correction; LBC: linear baseline correction; SNV: standard normal variate transformation; MSC: multiplicative scatter correction; SGD: Savitzky-Golay first derivative with a second-order polynomial and a 3-point window.

(purple circles) and non-fresh (gray circles) quail eggs. As shown, OFF (Figure 4a), OFF + LBC (Figure 4c), and SNV (Figure 4d) incorrectly projected only one out of 30 fresh quail egg sample outside the acceptance area, representing a sensitivity of 96.7% and a specificity of 100% (Table 1). In contrast, LBC (Figure 4b) incorrectly projected only one out of 246 non-fresh quail egg sample within the acceptance area, implying a sensitivity of 100% and a specificity of 99.6% (Table 1). On the other hand, MSC (Figure 4e) and SGD (Figure 4f) achieved a 100% sensitivity and specificity in the test set, projecting correctly all fresh and non-fresh quail egg samples inside and outside their respective acceptance areas. These last models were also the most parsimonious using only 7 principal components to reach the best predictive ability. These models also demonstrated the highest overall accuracy of 99.1%, with only three misclassifications from a total of 336 quail egg samples (Table 1).

Regarding the literature, our proposed work outperforms the overall accuracy (over 80%) obtained by Brasil *et al.*²¹ Moreover, the methodology is simplified since it is based exclusively on the chemical profiling contained in the NIR spectra, instead of using two different quality parameters (HU and EQI) as reference methods. Additionally, PLS-DA and SVM classifiers require *a priori* knowledge of at least two defined classes. Consequently, any new unknown sample in the prediction step will be assigned to a modeled class, regardless of its chemical or physical similarity. In contrast, with DD-SIMCA, new unknown samples are assigned as belonging to the target class based on the pattern of measured analytical signals from the target samples. This eliminates the need to construct various representative classes for the different levels of temperature and UV radiation exposure, for instance.

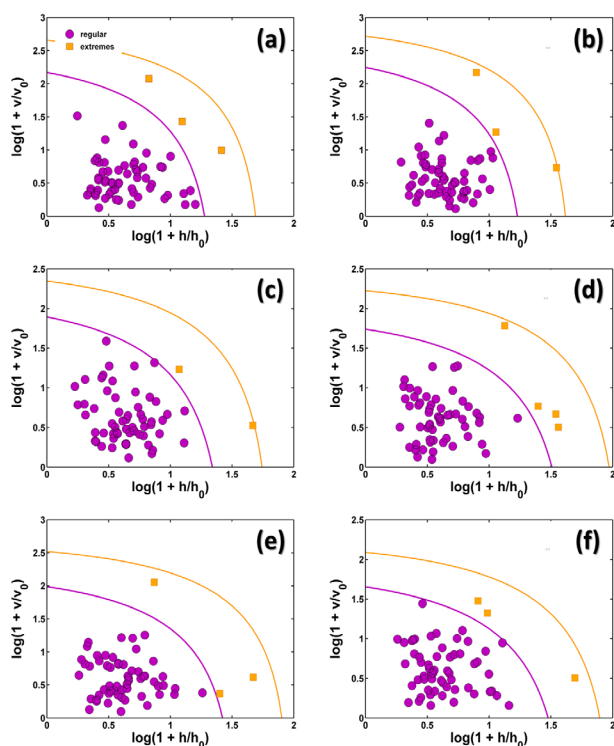


Figure 3. Acceptance areas obtained by DD-SIMCA for the authentication of fresh quail eggs using NIR spectra pre-processed with a 7-point moving window smoothing followed by (a) offset correction (OFF), (b) linear baseline correction (LBC), (c) OFF + LBC, (d) standard normal variate transformation (SNV), (e) multiplicative scatter correction (MSC), and (f) Savitzky-Golay first derivative with a second-order polynomial and a 3-point window (SGD). Fresh quail eggs (regular) and extreme samples are depicted in purple circles and orange squares, respectively. Purple and orange lines indicate the thresholds constructed for the acceptance and outlier detection areas, respectively.

Conclusions

This study highlights the potential of NIR spectroscopy for analyzing the freshness of quail eggs, offering qualitative insights on the influence of controlled conditions of temperature and UV radiation exposure during storage. Indeed, the influence of temperature and UV radiation provokes chemical and physical changes in the internal quality of the quail eggs, particularly due to water loss through the shell and changes in moisture content within the egg, as well as to potential structural changes in proteins, thus, resulting in an increase in the air chamber, degradation of the inner membrane, and distortion of the yolk symmetry. These changes are easily detected by a miniaturized NIR spectrometer and then modeled by DD-SIMCA, proving to be an effective, low-cost technology that can be used by producers and regulatory agencies to guarantee the safety and good quality of food products, reinforcing the principles of Green Food Analysis and the Egg Industry 4.0 concept.

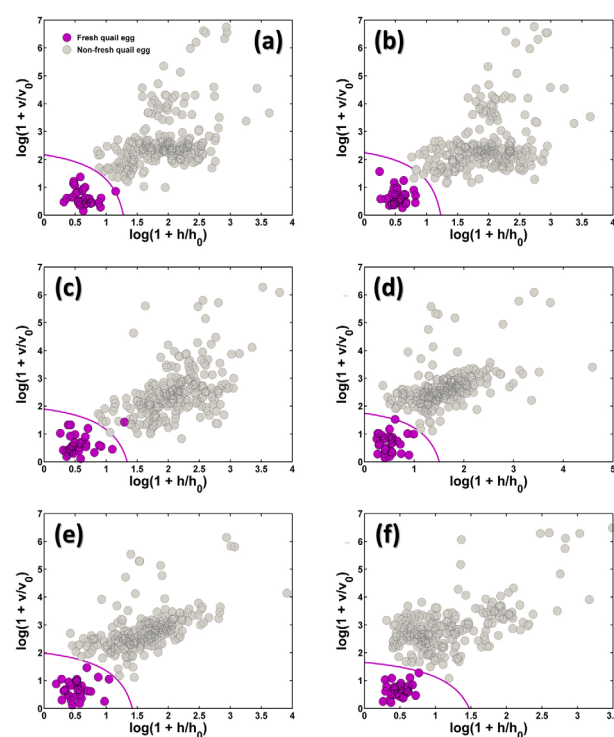


Figure 4. Projections of the test samples obtained by DD-SIMCA for the authentication of fresh quail eggs using NIR spectra pre-processed with a 7-point moving window smoothing followed by (a) offset correction (OFF), (b) linear baseline correction (LBC), (c) OFF + LBC, (d) standard normal variate transformation (SNV), (e) multiplicative scatter correction (MSC), and (f) Savitzky-Golay first derivative with a second-order polynomial and a 3-points window (SGD). Fresh quail eggs (target) and non-fresh samples are depicted in purple and gray circles, respectively. Purple line indicates the threshold constructed for the acceptance area.

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Author Contributions

Karoline M. Silva was responsible for formal analysis, validation, visualization, writing original draft; David Douglas S. Fernandes for software, formal analysis, validation, visualization, data curation, writing review and editing; Edilene D. T. Moreira for methodology, investigation, visualization, writing review and editing; Fernando G. Perazzo for methodology, investigation, visualization, writing review and editing; Wellington S. Lyra for methodology, investigation, visualization, writing review and editing; Paulo Henrique G. D. Diniz for conceptualization, data curation, supervision, funding acquisition, project administration, writing (original draft, review and editing).

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