

Analytical Methods

A quick method for fraud detection in egg labels based on egg centrifugation plasma

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ABSTRACT

The aim of this work was to develop a quick and cheap method for fraud detection in egg labels according to the four legal farming method of the EU. The plasma obtained from egg centrifugation was investigated for this purpose. Initial protein content in egg, plasma protein content, plasma colour parameters (L^* , a^* and b^*) and plasma UV–VIS–NIR (Ultraviolet–Visible–Near-infrared) spectra were evaluated. The classification algorithms applied were SVM (Support-Vector-Machine), LDA (Linear-Discriminant-Analysis) and QDA (Quadratic-Discriminant-Analysis). The analysis of the protein content did not detect differences. Colour parameters and spectral measurements showed significant differences between eggs. Spectra analysis with QDA gave sensitivity of 100% in the calibration set. The validation set scored 87.5% sensitivity and 94.07% specificity using the visible spectra. This work established plasma spectral measurements combined with classification algorithms as a powerful tool to discriminate the four farming systems. This work presents a fast tool for the egg label control.

1. Introduction

A previous work developed a method for fraud detection in hen housing system declared on the eggs' label. It was based on UV–VIS–NIR (Ultraviolet–Visible–Near-infrared) spectroscopy and chemometrics. The method used an egg yolk lipid extract and samples were classified with 100 % sensitivity (Puertas & Vázquez, 2019). The worthy results in addition to the relevance of eggs' label fraud aroused attention of business and consumers. Food companies were interested in that method because it made feasible to test their farms or suppliers. While it could give more trustworthiness among consumers.

There are four main systems for keeping laying hens in the EU. They were detailed by the Commission Regulation 589/2008 (EC). In 2018, half of the EU's hens were kept in enriched cages – cages equipped with perches, nests, scratching areas and nail shorteners. The other half were kept in cage-free egg production systems: either barns, free-range or organic rearing systems with around 28 %, 16 % and 5 %, respectively. Barns are large enclosures with litter on the floor and freedom of movement for the birds within the poultry house. Free-range systems are like barn systems with access to an outdoor run (Augere-Granier, 2019). Organic systems are a specific form of free-range hens where they also have access to a pasture and obey the organic production principles such as feed ingredients grown without synthetic fertilizers and no products

from genetically modified organism (European Commission, 2008). These four farming systems are coded according to the Commission Directive 2002/4/EC: 0- for organic production, 1- for free range, 2- for barn and 3- for cages.

Consumers are raising awareness about animal welfare. European citizens' initiatives as "End the Cage Age" show this perception (European Commission, 2021). And eggs are today the unique food with a mandatory system in the European Union informing consumers on the label about the welfare conditions under which animals are kept (Simonin & Gavinelli, 2019). This has supposed an increasing demand of eggs labelled as 0 or 1 were laying hens have higher levels of welfare than barn eggs or eggs from caged hens. The higher production costs of these eggs make them more expensive. This arise interest of fraudsters because their potential financial gain is greater (Puertas & Vázquez, 2019). For example, in 2019, a part of the Operation OPSON VIII (a joint Europol/Interpol initiative targeting trafficking in fake and substandard food and beverages) was focused on the organic claim. The operation looked at organic food products and the growing trend of products falsely claiming to be organic to be sold at higher prices. Eggs were one of the food items under suspicion. In Spain, the police Guardia Civil seized more than 45.000 eggs incorrectly label (Interpol & Europol, 2020).

Nowadays, the authorities' procedure to confirm the hen housing

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method on the egg label is the traceability system of the eggs back to the farms of origin. In 2017, at UE level, this system was evaluated and overall succeed on, despite considerable difficulties. The fraud incident leading to the contamination of chicken eggs and egg products by the insecticide fipronil launched the system. Nonetheless, certain factors entailed a delayed response to this incident (European Commission & Directorate-General for Health and Food Safety, 2021). A quickest response is a challenge in the management of a food fraud case. On the other hand, authorities are concerned about the challenges that organic food traceability poses across all Member States. As evidenced by the Directorate-General for Health and Food Safety audits conducted in several nations (Directorate-General for Health and Food Safety, 2017, Directorate-General for Health and Food Safety, 2018, Directorate-General for Health and Food Safety, 2021; Health and Consumers Directorate-General, 2013). Reports revealed gaps in the system that malicious people may take advantage.

Since our first method was published, several articles had disclosed more spectroscopic methods to detect fraud in different food. The growing interest in these non-targeted fingerprinting techniques may be attributable to advances in the analytical tools as well as increased knowledge in the food industry and research on the benefits of using such techniques (Hassoun et al., 2020). For example, Visible-NIR spectroscopy has been employed to identify and quantify adulterants in honey or meat. NIR alone has demonstrated same applicability in milk and fish (Hassoun et al., 2020). A method combining ATR-FTIR (attenuated total reflectance Fourier transform infrared), NIR spectroscopy and chemometrics was established to detect liquid egg authenticity and adulteration with water (Uysal & Boyaci, 2020). Recently, a method based on NIR spectroscopy managed to classify egg white and yolk according to the system of production in cage or free range with a portable device (Hoffman, Ni, Dayananda, Ghafar, & Cozzolino, 2022). Other methods described in literature for egg classification employed ¹H NMR or HPLC combined with chemometrics (Ackermann et al., 2019; Campmajó, Cayero, Saurina, & Núñez, 2019; Hajjar, Haddad, Rizk, Akoka, & Bejjani, 2021).

Yolk colour is the most important physical egg characteristic for consumers (Berkhoff et al., 2020). The yellow-orange colour of egg products is due to the carotenoids in the yolk, mainly carotene and xanthophylls (lutein, cryptoxanthin, and zeaxanthin). As hens cannot synthesize carotenoids, the feed is their only source of these compounds. The close relation between carotenoids in the diet, the deposition of xanthophylls in the egg yolk and correlation with the resulting yolk colour has been extensively reviewed (Hammershøj & Johansen, 2016). Several studies have been done to classify the four farming methods according to egg yolk colour. Even egg yolk colour showed to be the sole sensory trait distinguishing eggs from organic farms (Dalle Zotte et al., 2021). Because it is highly affected by plant material type and intake of the hen (Hammershøj & Johansen, 2016). Colour has been measured in the form of carotenoids composition or with the colour parameters: L*, a*, b* or Roche yolk colour fan (Dalle Zotte et al., 2021). In this study, plasma colour parameters L*, a*, b* were obtained from spectral measurements. Because carotenoids absorb at the end of the visible range, from around 470 to 380 nm (Puertas & Vázquez, 2020).

The aim of this work was to develop a quicker and cheaper method for fraud detection in egg labels. Spectra from centrifugation plasma combined with chemometric algorithms were evaluated as a powerful tool to discriminate the four farming systems. Additionally, protein content and colour parameters were also evaluated as potential identifiers of the hen farming method.

2. Materials and methods

2.1. Samples distribution

A total of 84 eggs were bought from local supermarkets. Three different suppliers were employed for each farming system and at least

six eggs from every farm were studied. The number of organic eggs (code 0) and free-range eggs (code 1) analysed was 18 for each system. While 24 barn eggs (code 2) and 24 eggs from caged hens (code 3) were evaluated.

2.2. The method

The process flowchart is shown in Fig. 1. Each egg was homogenised with a high-performance homogenizer (Ultra Turrax®, IKA, Staufen, Germany) at 10,000 rpm for 1 min and centrifuge for 30 min at 13,273g (Spectrafuge™ 24D Microcentrifuge, Labnet International, Inc, New Jersey, USA). Because the goal was to have the highest plasma-granule separation in the shortest time, these settings were chosen based on previous research (Puertas & Vázquez, 2021b). Then plasma was separated from granules with an appropriate pipette Pasteur. The UV-VIS-NIR spectra of each plasma was measured with Spectrophotometer Jasco V670 (Jasco Inc., Hachioji, Tokyo, Japan). The cuvette employed is made of Quartz Suprasil® 300 with a 1 mm light path (Hellma GmbH & Co. KG, Mulheim, Germany). UV-VIS-NIR spectra were acquired in transmittance mode (T) at 2 nm intervals in the range of 190–2500 nm. Then a total of 2311 variables were acquired. Samples were measured at 22–24 °C. UV region was considered from 190 to 380 nm, VIS region from 380 to 780 nm and NIR region from 780 nm up to 2500 nm (Porep, Kammerer, & Carle, 2015). Each plasma sample was measured twice making a total of 168 UV-vis-NIR spectra. Spectral data were collected with Spectra Manager™ II software (Jasco Inc., Hachioji, Tokyo, Japan).

2.3. Protein and colour determination

Proteins from initial egg and plasma were measured with Nanodrop 2000 (Thermo Scientific™, Waltham, MA, USA). The method followed is described elsewhere (Puertas & Vázquez, 2021a). Protein concentration was estimated by the Warburg and Christian method (1942) based on the distinct ultraviolet light absorption maximum at 280 nm that most proteins exhibit. Results are expressed in mg of protein by 1 ml of egg/plasma. To eliminate the interference of nucleic acids in the estimation of protein content, this equation was used (Kalckar, 1947):

$$\text{Protein concentration (mg/ml)} = 1.45 \times A_{280} - 0.74 \times A_{260} \quad (1)$$

where A_{280} and A_{260} are the absorbance values obtained at 280 nm and 260 nm, respectively. Nucleic acids have also a strong ultraviolet absorption band at 280 nm. However, their absorption is much more strongly at 260 nm than at 280 nm, whereas with proteins the reverse is true. When samples have a ratio $A_{280}/A_{260} < 0.6$, the content of nucleic acids is more than 20 % and the method gives considerable errors. This ratio was supervised for each sample.

The colour of the samples was studied through the L*, a* and b* parameters obtained from each spectrum. The CIE parameters were determined by the Spectra Manager™ II software (Jasco Inc., Hachioji, Tokyo, Japan) established on the 2° standard observer with light source D65 (Vázquez, Velazquez, & Cazón, 2021). Each sample got two replicates. The parameter L* measures the degree of lightness (0 = black, 100 = white). The a* coordinate expresses redness (−100 = green, 100 = red) and b* reflects yellowness (−100 = blue, 100 = yellow) (Hammershøj & Steinfeldt, 2015).

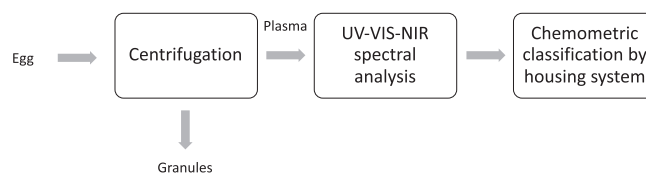


Fig. 1. Flowchart of the proposed quick procedure for classification of egg by the four housing systems by UV-VIS-NIR spectral analysis.

2.4. Data processing and statistical analysis

UV–vis–NIR spectra were exported from Spectra Manager™ II software to Unscrambler® software Version 10.5 (Camo, Oslo, Norway) for chemometric analysis. Tukey HSD test was performed on the protein and colour data obtained.

Principal component analysis (PCA) was carried out on spectral data to reduce the data dimensionality and to retain as much as possible the information present in the data (Tres & Van Ruth, 2011). PCA allows to detect outliers, recognize patterns in samples distribution and relationships between variables and classes (Ríos-Reina, García-González, Callejón, & Amigo, 2018). The capability and effectiveness of PCA for accurate sample classification is very limited (Bwambok et al., 2020). To address these challenges Support Vector Machine classification (SVM), Linear and Quadratic Discriminant Analysis (LDA and QDA) were employed for sample differentiation and classification. These are supervised classification techniques, because the number of categories and samples in each category are previously defined.

SVM is a pattern recognition method that defines a function to describe the limit to separate classes by maximizing the distance between them. It aims to construct an optimal separating plane by mapping the original data points from the input space into a high-dimensional feature space. It employs kernel functions to map from the original space to the feature space. The distances from all the data points to the separating plane are minimum. If a point is situated on the wrong side of the margin, the margin is maximized by penalizing the point. Support vectors are the points on the boundary or on the wrong side of the margin supporting the split (Martelo-Vidal, Domínguez-Agís, & Vázquez, 2013; Yang et al., 2018; Yi et al., 2016). In the present study, cross validation was performed to analyse SVM models.

LDA produces a linear decision boundary between two classes, which maximizes the ratio between-class variance and minimizes the within-class variance. The number of discriminant functions for classify 4 groups is 3. Whereas the boundary produced by QDA is a quadratic curve, which, unlike LDA, may consist of two separate sections of boundary lines. This means it is thus able to correctly classify samples from a class even if they lie in separate regions in variable space. At LDA the number of samples must be larger than the number of variables, allowing the calculation of the inverse of the variance–covariance matrix. Then a spectral data reduction is needed. For that purpose PCA is employed; reducing the large multivariate data matrix down to a few principal components (PC), which still contain the majority of the information held in the original raw data (Dixon & Brereton, 2009). The number of PC would still need to be less than the number of objects.

For each classification method, several analyses were performed dividing the variable set. The spectra were studied complete (2311 variables) and divided in UV (191 variables), VIS (400 variables), NIR (1720 variables), UV–VIS (591 variables) and VIS–NIR (2120 variables). Whereas protein and colour results were analysed together. Methods were evaluated in terms of sensibility and specificity. For each variable set, the method with the best results was tested through a full model validation using a test set. Sample set was split into a calibration subset and validation set, 75 % and 25 % respectively. Calibration subset was used to generate the models and the validation set was used to test the calibrations (Manuelian, Currò, Penasa, Cassandro, & De Marchi, 2017). The Ms-Excel random function was employed to randomize the set.

3. Results and discussion

3.1. Spectra analysis

PCA analysis revealed that 98 % of the raw data was explained with 2 components (Fig. 2). In addition, two samples were detected as outliers, one from barn eggs and one from caged hen. These samples caused the four points outside the Hotelling's T^2 ellipse shown at Fig. 2. Hereinafter the sample size was reduced to 82 and 164 spectra. Deprived of outliers, barn eggs and most of the organic eggs obtained negative values for PC-1, opposite to free-range eggs that got positive values. All of them had positive and negative PC-2 scores. Rather, eggs from caged hens were spread across the ellipse, but most of them obtained positive PC-2 scores. Despite this, the considerable overlap of these scores highlights the need for a different classification model to distinguish the four farming methods.

The UV–VIS–NIR spectra from all these samples are shown at Fig. 3. Spectra were colour classified by farming method. In contrast to the spectra obtained from saponified samples at our first method, the transmittance is null at more points. Due to the absence of an extraction step, there are more compounds at the samples that can interrupt the light pathway. The highest variability is also detected at the VIS spectrum. However, short-wavelengths in the NIR region (1300–780 nm) evidenced considerable differences between sample transmittances.

3.2. Protein and colour results

Neither in egg, nor in plasma were detected significant differences between the four farming methods by the protein content. Results are shown on Table 1. The average values were 142.04 ± 7.02 and 90.77 ± 5.82 mg/ml for egg and plasma, respectively. These values showed that

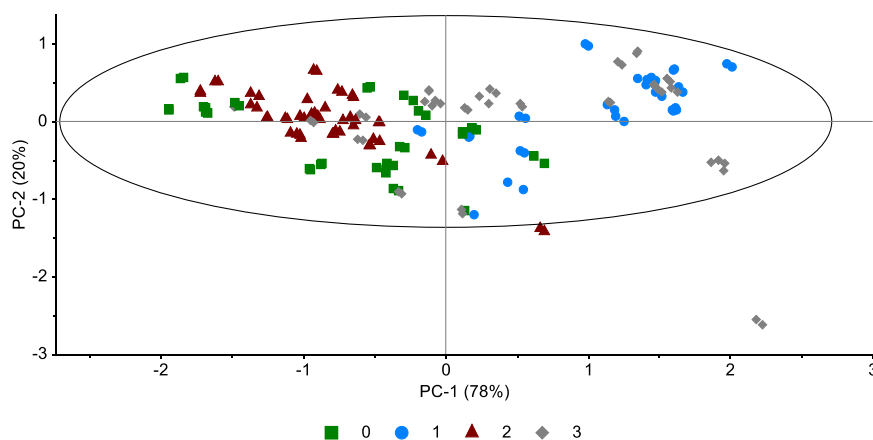


Fig. 2. PCA results of UV–VIS–NIR spectra from the 84 samples. Hotelling's T^2 ellipse with a significance level of 5%. In green from organic eggs, in blue from free-range hens, in brown from barn eggs and in grey from eggs of caged hens. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

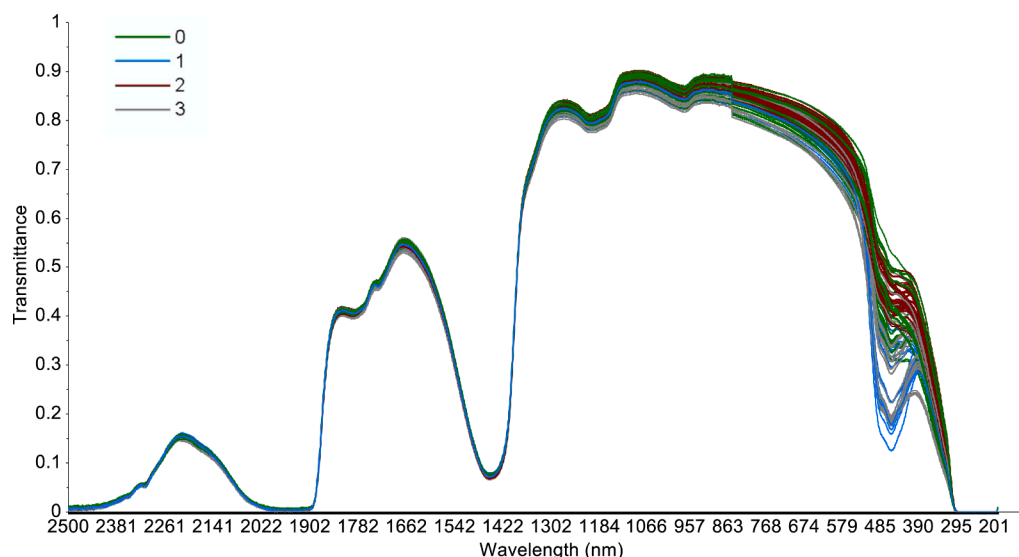


Fig. 3. UV–VIS–NIR transmittance spectra. In green from organic eggs (0), in blue from free-range hens (1), in brown from barn eggs (2) and in grey from eggs of caged hens (3). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

Table 1

Protein content and colour results. Organic eggs (0), free-range hens (1), barn eggs (2) and eggs of caged hens (3).

Parameter	Farming method				Average
	0	1	2	3	
Egg protein (mg/ml)	143.92 ± 8.33 ^a	138.71 ± 5.58 ^a	142.62 ± 5.63 ^a	142.61 ± 7.70 ^a	142.04 ± 7.02
Plasma protein (mg/ml)	91.41 ± 5.58 ^a	88.28 ± 2.85 ^a	92.28 ± 5.61 ^a	90.72 ± 7.42 ^a	90.77 ± 5.82
L*	86.07 ± 1.79 ^a	84.06 ± 0.76 ^b	86.59 ± 0.93 ^a	84.48 ± 1.60 ^b	85.33 ± 1.69
a*	-0.99 ± 0.73 ^b	-1.02 ± 0.77 ^b	0.36 ± 0.42 ^a	0.33 ± 0.83 ^a	-0.25 ± 0.96
b*	24.61 ± 5.49 ^c	44.80 ± 10.55 ^a	23.50 ± 2.19 ^c	36.49 ± 11.75 ^b	32.06 ± 11.97

Values with different letters are significantly different ($p < 0.05$).

around 63.9 % of the whole egg proteins were in the plasma recovered. This value is consistent with previous works (Puertas & Vázquez, 2021a). Within the centrifugation conditions applied (13273 g for 30 min), plasma made up approximately 79 % of total whole egg (Puertas & Vázquez, 2021b). The expected composition in dry matter of the plasma employed was around half proteins, half lipids (Puertas & Vázquez, 2021a).

Our results in egg protein content with non-significant differences between the rearing systems are opposite to previous works. Hidalgo et al. (2008) obtained less proteins in eggs from cage hens. While other studies obtained the lowest protein contents in organic eggs (Dalle Zotte et al., 2021; Rakonjac et al., 2018). These differences evidence that egg protein content alone cannot classify the four kinds of eggs.

In contrast, colour parameters presented differences among the four farming methods (Table 1). The high standard deviation of the positive parameter b*, around 12, demonstrate disparities on yellowness between the eggs from the four farming methods. The plasmas of free-range eggs were the most yellowish, caged eggs obtained intermediated values while organic and barn eggs got similar b* values. The lightness of these eggs coded 0 and 2 is also similar. But parameter a* can differentiate them with negative average value for organic eggs and positive for barn eggs. Although they are all close to 0. These a* results showed that plasmas were yellow rather than orange-coloured. The approach of assessing the hen farming method through the plasma

obtained from whole egg centrifugation has never been considered. Then the values of plasma colour parameters and protein content cannot be compared.

3.3. Calibration results

SVM was applied over the full and divided UV–VIS–NIR spectra. Linear kernel function was set for spectral data because it is suitable when the number of variables is very large (2311 variables). In contrast, radial basis function was used for protein and colour parameters since only 3 or 4 variables were analysed; corresponding to L*, a*, b* alone or with protein content value. Table 2 show the results of this classification method. The percentage of properly classified samples in the training set is indicated in each analysis. The best training and cross validation sensitivities obtained from spectral data were 84.76 % and 81.10 %, respectively. This meant that more than 80 % of the samples were correctly predicted. These were achieved with only VIS–NIR data. It classified properly around 95 % of the organic and barn eggs. However, protein and colour parameters obtained 100 % sensitivity and specificity on training set and sensitivity values over 90 % from cross validation test.

Results from LDA are shown at Table 2. The spectra were also analysed complete and partially divided, but only classification results over 95 % sensitivity are shown. With LDA, organic eggs were always classified with 100 % sensitivity. Lower sensitivities were obtained combining colour and protein parameters, egg proteins did not modify the sensitivity of the method. LDA obtained the highest sensitivity (98.78 %) with two models. The model that employed the whole spectra and the model that employed only UV–VIS. LDA with 2311 variables from UV–VIS–NIR and 20 PC classified eggs from organic farms and caged hens with 100 % sensitivity. While LDA with only UV–vis data (591 variables) and 18 PC categorised 100 % all types except free range eggs. The UV–VIS–NIR model reached the highest specificity value of 99.39 %, then <1 % of all samples were predicted incorrectly.

On the other hand, QDA and spectral data were able to categorize all the samples with 100 % sensitivity. Table 2 shows the results. UV–VIS data with 6 PC was the spectrum region with the fewest principal components to that goal. As NIR data did not achieve 100 % sensitivity, results are not included. Similar results over 92 % sensitivity were obtained if colour parameters are analysed alone or combined with protein content. Nonetheless, these parameters did the best classification with SVM.

Table 2

Calibration results. Organic eggs (0), free-range hens (1), barn eggs (2) and eggs of caged hens (3).

SVM	Kernel type	Sensitivity (%)		Types of eggs correctly predicted (%)				Specificity (%)
		Training	Cross validation	0	1	2	3	
UV-VIS-NIR	Linear	81.1	81.71	88.89	72.22	95.65	67.39	91.94
UV	Linear	64.02	65.85	86.11	66.67	78.26	30.43	86.95
VIS	Linear	82.93	80.49	94.44	72.22	95.65	69.57	92.12
NIR	Linear	80.49	57.93	75.00	77.78	82.61	84.78	93.46
UV-VIS	Linear	81.71	79.27	94.44	72.22	95.65	65.22	92.15
VIS-NIR	Linear	84.76	81.10	94.44	77.78	95.65	71.74	93.15
Proteins & colour	RBS	100	94.51	100	100	100	100	100
Plasma proteins & colour	RBS	100	90.854	100	100	100	100	100
Colour	RBS	95.73	92.68	94.44	91.67	100	95.65	91.94
LDA	No. PC	Sensitivity (%)		0	1	2	3	Specificity (%)
UV-VIS-NIR	20	98.78		100	97.22	97.83	100	99.39
VIS	13	95.12		100	88.89	95.65	95.65	97.56
UV-VIS	18	98.78		100	94.44	100	100	99.19
VIS-NIR	19	97.56		100	97.22	100	93.48	98.98
Proteins & colour	na	83.54		100	80.56	86.96	69.57	93.36
Plasma proteins & colour	na	83.54		100	83.33	89.13	65.22	93.57
Colour	na	80.49		100	77.78	91.30	56.52	92.15
QDA	No. PC	Sensitivity (%)		0	1	2	3	Specificity (%)
UV-VIS-NIR	11	100	100	100	100	100	100	100
UV	12	100	100	100	100	100	100	100
VIS	9	100	100	100	100	100	100	100
UV-VIS	6	100	100	100	100	100	100	100
VIS-NIR	1	100	100	100	100	100	100	100
Proteins & colour	na	92.68	100	91.67	97.83	82.61	82.61	96.93
Plasma proteins & colour	na	92.68	100	100	97.83	76.09	76.09	97.55
Colour	na	92.07	100	88.89	100	80.43	80.43	96.53

RBS: radial basis function; na: not applicable.

3.4. Validation results

The 82 samples were divided in two groups: 62 for model calibration and 20 for validation. The randomization resulted in 5 organic eggs, 4 free range eggs, 5 barn eggs and 6 eggs from caged hens at the validation set. The average egg and plasma protein contents for the calibration subset were 141.99 ± 6.81 mg/ml and 90.71 ± 5.23 mg/ml, respectively. While the validation set got 142.19 ± 7.64 mg/ml and 90.96 ± 7.36 mg/ml on egg and plasma proteins, respectively. The average values for the colour parameters on the calibration subset were: 85.41 ± 1.60 for L^* , -0.27 ± 0.93 for a^* , 31.64 ± 11.67 for b^* . While the averages at the validation set were 85.08 ± 1.95 for L^* , -0.18 ± 1.05 for a^* and 33.37 ± 12.80 for b^* .

The validation analysis was performed with QDA for the spectral data and SVM with the colour and protein content data; since these models obtained the best results with those variables. Table 3 shows the results. Except for colour parameters, all calibration models obtained 100 % sensitivity. When the model was used for allocating new and unknown samples to the most probable class, the sensitivity decreased up to 55 %. The best validation results were achieved with spectrum

data rather than the values of protein and colour parameters. QDA and the visible spectrum region were the best combination to predict the farming method. They achieved a sensitivity of 87.5 % and specificity of 94.07 % in the validation set. Barn eggs and eggs from caged hens were classified with 100 % and nearly 92 % sensitivity, respectively.

Low sensitivity was obtained at classification models employing plasma and whole egg protein content. While sensitivity increased from 55 % to 80 % when only colour parameters were employed. These results support that colour parameters are significantly different between the four husbandry systems. It is noteworthy that similar values for two colour parameters (L^* and b^*) were obtained for eggs coded 0 and 2. Their plasmas were less yellowness and brighter than those from eggs coded 1 or 3. When egg is centrifuged, carotenoids are separated between plasma and granules according to centrifugation conditions (Puertas & Vázquez, 2021b). With the conditions employed in this work, it seems that yellowish carotenoids from whole egg remained at plasma, while mostly of the reddish pigments precipitated with granules. From literature, it is described that dietary lutein contributed to yolk yellowness, while zeaxanthin, β -cryptoxanthin and β -carotene contributed to yolk redness (Kljak et al., 2021). Nevertheless, further analyses are

Table 3

Validation results. Organic eggs (0), free-range hens (1), barn eggs (2) and eggs of caged hens (3).

Variables	Method	No. PC	Sensitivity (%)		Types of eggs correctly predicted (%)				Specificity (%)
			Calibration	Validation	0	1	2	3	
UV-VIS-NIR	QDA	11	100	75	60	62.5	90	83.33	88.79
UV	QDA	12	100	70	100	25	50	91.67	85.25
VIS	QDA	9	100	87.5	80	75	100	91.67	94.07
UV-VIS	QDA	6	100	82.5	100	38	80	100	90.00
VIS-NIR	QDA	11	100	75	50	50	90	100	88.24
Proteins & colour	SVM	na	100	55	40	50	80	50	81.82
Plasma proteins & colour	SVM	na	100	55	40	100	20	66.67	85.00
Colour	SVM	na	98.39	80	60	75	100	83.33	91.53

na: not applicable.

needed to identify these carotenoids in plasma from whole egg centrifugation. The disparity in those reddish pigments that remains in plasma explained the difference between barn and organic eggs.

However, classification results from validation obtained from UV–VIS or VIS spectra were over the 80 % obtained with colour parameters. UV–VIS and VIS spectra achieved sensitivities of 82.5 % and 87.5 % and specificities of 90 % and 94.07 %, respectively. Consequently, considering all spectra is better rather than only colour parameters to develop these classifications models.

In this study the plasma obtained from whole egg centrifugation was employed. Compared to the egg yolk lipid extract employed at the first method, separation of yolk from whole egg is not needed and the three hours of saponification time is reduced to 30 min of centrifugation time. The new method developed avoid solvents and filtration; therefore, sample manipulation time and consumable costs are reduced. Spectral analysis and chemometric classification are steps shared between both methods.

Plasma from whole egg was chosen to classify the four farming methods because it contains lipids and proteins from hen diet. Several studies have analysed different factors, such as hen diet, age or breed, to impact on the composition of the egg. Various studies conclude that hen diet is more influential than heredity or strain in its effect on variability of egg composition (González-Muñoz et al., 2009; Washburn, 1979). On the other side, it is described that each housing system supposes clearly differences in hen feed. Organic and free-range hens have access outdoors, thus they are exposed to sunlight, and they have access to other food sources such as worms, insects or seeds from vegetation. But feeds supplied to organic hens should be organic. While barn and caged hens have less access to other food sources and their feed can contain chemical additives for example synthetic xanthophylls that are banned in organic feed (Dalle Zotte et al., 2021). These variabilities in hens diet result in eggs with different nutrients composition. Beside each egg sample has a unique composition due to the intrinsic metabolism of each chicken that originated it.

Yolk colour can be controlled through hen feed, and it only represent carotenoids composition in yolk, as aforementioned. Then differences between the four legal farming system should not be settled only based in this parameter. An analytical tool is needed. This work established plasma spectral measurements combined with classification algorithms as a powerful tool to discriminate the four farming systems.

Since our first method to detect fraud at egg label was published, new studies have described differences between organic eggs and other rearing systems. Ackermann et al. (2019) developed a prediction model that correctly classified 93 % of the organic eggs. In this study, ¹H NMR of freeze-dried egg yolk samples were analysed using PCA followed by LDA. Dalle Zotte et al. (2021) detected that farming method affected physicochemical and sensory traits of organic, barn and cage eggs. One work studied eggs from cages hens, free-range, organic and family farms. It build classification models with high-resolution ¹H NMR (Nuclear Magnetic Resonance) of triacylglycerols and chemometrics (Hajjar et al., 2021). Recently, a portable NIR spectroscopy was evaluated for classification of cage and free-range eggs. Egg white and egg yolk were directly examined and achieved a sensitivity of 86 % with LDA (Hoffman et al., 2022). The absence of granules in plasma herein produced higher sensitivities in calibration and validation models. Nevertheless, only another method has been published to classify eggs in the four legal farming methods. Samples were analysed through high-performance liquid chromatography with ultraviolet detection (HPLC-UV) fingerprints which were subsequently processed by means of PCA and partial least squares-discriminant analysis (PLS-DA). This method reached a classification rate of at least 82.6 %, with 100 % for organic vs non-organic hen eggs (Campmajó et al., 2019). The last two methods cover complex sample extraction and data analysis, combine with costly and tough equipment.

The method described herein obtained a total validation sensitivity of 87.5 % with 100 % sensitivity for barn eggs. Visible range and QDA

obtained the best results. These good scores pair with a method straightforward, rapid and economical. Thus, this work presents a screening tool reliable, fast and affordable that could perform egg label control and give consumers trust in food chain while dissuades fraudsters.

4. Conclusion

The plasma obtained after whole egg fractionation by centrifugation is useful to identify the farming method. Results demonstrated that protein content does not detect differences between the four farming methods. Colour parameters and spectral measurements showed significant differences between classified eggs. However, colour parameters provide less evidence to properly classify eggs according to the husbandry systems. Spectra analysis with QDA gave a higher accurate categorisation of the four legal husbandry systems. Visible spectral range obtained 87.5 % sensitivity at validation set supported by 100 % sensitivity at calibration set. This rapid method reduces time at laboratory steps for manipulation and results interpretation. Therefore, it entails an enhancement of the response time to a food fraud incident.

CRedit authorship contribution statement

Gema Puertas: Investigation, Methodology, Writing – original draft. **Patricia Cazón:** Validation, Writing – review & editing. **Manuel Vázquez:** Conceptualization, Writing – review & editing.

Declaration of Competing Interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: Gema Puertas reports financial support was provided by Spanish Ministry of Education, Culture and Sport.

Data availability

Data will be made available on request.

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