doi:10.1017/S0043933919000424

Potential use of spectroscopic techniques for assessing table eggs and hatching eggs

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In evaluating the quality of table eggs and the developmental stages of embryonic eggs, spectroscopic techniques provide greater efficiency than traditional, time-consuming and laborious approaches. This review summarises recent developments in the spectroscopic analysis of table eggs, including the determination of the chemical composition (ratios of performance to standard deviation of 4.38, 2.25, 2.28, 2.31, and 3.03 for fat, moisture, and protein in egg yolk and moisture and protein in egg albumen, respectively, have been reported). A Haugh unit detection accuracy RMSEP (root mean square error of prediction) for quality of 6.29 was obtained by hyperspectral imaging) for table eggs and fertility detection (for white-shell eggs, fertility detection has been realised at a promising rate of 93.5%) and gender determination in hatching eggs. In conclusion, hyperspectral imaging generally outperforms visible or near-infrared reflectance spectroscopy when evaluating both consumption eggs and hatching eggs, and near-infrared reflectance Raman and fluorescence spectroscopy exhibit a strong potential for gender determination prior to hatching. Scientists have attained a correct sexing rate above 90% at 3.5 d of egg incubation without removing the inner shell membrane. In the detection of blood-spot eggs or fertile eggs, eggshell colour proved to be a negative factor.

Keywords: table egg; hatching egg; quality; spectroscopic technique

Introduction

Eggs are a popular source of nutrition as they contain several nutrients, which are essential for humans, namely, water (~75%), proteins (~12%), and lipids (~12%) (Burley and Vadehra, 1989; Sugino *et al.*, 1996). However, the nutrient content of eggs varies depending on breed and feeding regime. Moreover, the internal quality of eggs can change during storage and transport (Silversides and Scott, 2001). Thus, it is

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Received for publication September 16, 2018
Accepted for publication June 11, 2019

https://doi.org/10.1017/S0043933919000424 Published online by Cambridge University Press

important to determine the chemical composition when discriminating between eggs of different quality.

For breeder eggs, the hatching rate is a critical economic factor for poultry farms. Infertile eggs or embryonic death occur in more than 10% of the eggs in hatcheries (Smith *et al.*, 2008). These eggs pose problems for the poultry industry because they occupy space, increase handling costs, and have the potential to harbour bacteria which could contaminate other eggs. Therefore, the early detection of non-fertile eggs and early and late embryonic death would increase the overall yield of the poultry industry.

There are many traditional methods for analysing nutrient components, quality, and embryonic death in chicken eggs, such as the Soxhlet extraction for crude fats (Song *et al.*, 2000), Kjeldahl method for crude protein (Lunven *et al.*, 1973), Haugh unit (HU) method for egg quality (Haugh, 1937), and candling for removing infertile eggs and dead embryos from hatching eggs. However, these methods are time consuming and unsuitable for in-line applications.

Recently, spectroscopic techniques as non-destructive, rapid and safe approaches have been successfully applied to the quantitative determination of major egg constituents (Dalle Zotte *et al.*, 2006; Zhao *et al.*, 2018). Most studies on these applications have assessed the quality of eggs and their products in terms of storage time (Xu *et al.*, 2014; Coronel-Reyes *et al.*, 2018), HU (Lin *et al.*, 2011; Suktanarak and Teerachaichayut, 2017), air cell height (Aboonajmi and Najafabadi, 2014; Aboonajmi *et al.*, 2016), and albumen pH (Abdel-Nour *et al.*, 2011; Dong *et al.*, 2017a). The detection of infertile and non-hatchable eggs has been investigated using spectroscopic methods (Lawrence *et al.*, 2007; Smith *et al.*, 2008; Liu and Ngadi, 2013). Furthermore, several studies have explored the feasibility of spectral analysis for the sexing of chicken eggs (Göhler *et al.*, 2016; Galli *et al.*, 2016; 2017).

An excellent review of spectroscopic techniques expounded on their ability to determine the quality of eggs (De Ketelaere *et al.*, 2004). This paper summarises developments published after 2004, taking some new parameters of eggs and their products into consideration.

Applications for determining chemical composition

Conventional techniques to determinate the fat, protein, and moisture content in eggs are expensive and can contaminate the samples (Lunven *et al.*, 1973; Song *et al.*, 2000). Wehling *et al.* (1988) used near-infrared reflectance (NIR) spectroscopy to measure protein and fat in spray-dried whole eggs based on three wavelengths. However, the small number of training samples again limited the applicability of this method. The use of near-infrared spectroscopy to determine the fat, moisture, and protein content in homogenised egg yolk and egg albumen (Zhao *et al.*, 2018) was recently reported and found that the evaluation of protein was not as accurate as that of fat but was superior to that of moisture.

Eggs are popular because of the n-3 fatty acids contained in the yolk, which can decrease the incidence of cardiovascular disease and are essential to human health. To guarantee that consumers receive appropriate amounts of these fatty acids, scientists applied hyperspectral imaging to predict the n-3 fatty acid content of eggs (Abdel-Nour and Ngadi, 2011), achieving R^2 values of 0.89, 0.54, and 0.75 for alpha-linolenic acid (ALA), eicosapentaenoic acid (EPA), and docosahexaenoic acid (DHA), respectively. In a previous study (Dalle Zotte *et al.*, 2006), NIR spectroscopy was exploited to determinate ALA, EPA, and DHA contents in egg yolk, and improved R^2 values of 0.96, 0.86, and 0.87 were reported, respectively. Both studies found that ALA

https://doi.org/10.1017/S0043933919000424 Published online by Cambridge University Press

could be predicted most accurately. Among all studies, fat has been modelled better than protein, which supports the conclusion that the quantitative detection of fats is generally better than that of protein when evaluating the near-infrared bands (Sasic and Ozaki, 2001).

Applications for assessing egg quality

For table eggs, consumers are particularly concerned with quality and freshness. Parameters such as shell quality, freshness, and blood spots are commonly used to assess egg quality.

EGGSHELL QUALITY

The eggshell provides natural protection for the egg and must be strong enough to prevent cracks from occurring during the packing and transportation processes. Traditional crack detection methods mainly rely on manual methods such as visual inspection or listening for a dull, flat sound as an indicator of a crack when tapping eggs together (Lawrence *et al.*, 2008). However, very small cracks, or micro-cracks, can go undetected by manual techniques. Narushin *et al.* (2004) applied infrared spectroscopy to predict eggshell quality parameters, such as shell weight, percentage, thickness, fracture force, maximal deformation and stiffness, but this method exhibited a poor predictive ability (correlation coefficients ranging from 0.29 to 0.72). In another study (Xiong *et al.*, 2013), eggshell percentage, strength, and thickness were analysed using NIR spectroscopy with partial least-squares regression (PLSR). Recently, Dong *et al.* (2017b) evaluated eggshell thickness based on visible (VIS)/NIR spectroscopy and reported a correlation coefficient of 0.86.

Once eggs have been cracked due to poor eggshell quality, they should be identified and removed before being sent to the market. Xiong *et al.* (2011) combined NIR and discriminant analysis (DA) to detect cracked eggs, achieving a fairly good recognition rate of 90%. Hyperspectral imaging combined with partial least squares-DA (PLS-DA) has been used to identify cracked eggs, outperforming the NIR model and achieving a classification accuracy of 97.06% (Xie and He, 2016).

EGG FRESHNESS

Freshness makes a major contribution to the internal quality of eggs. The traditional method of assessing egg freshness is the HU method (Haugh, 1937), which requires the eggs to be broken. Recently, spectroscopic techniques have been applied to determine the freshness of eggs. A new algorithm based on support vector data description (SVDD), which overcomes the problem of unbalanced samples, has been employed to identify fresh and non-fresh eggs in terms of HU, achieving an identification rate of 93.3% (Zhao *et al.*, 2010). Three variable selection methods (spectral interval selection, genetic algorithm (GA), and successive projections algorithm (SPA)) were combined with graphically oriented local multivariate analysis to identify egg freshness based on NIR spectroscopy (Lin *et al.*, 2015). Kemps *et al.* (2007) combined VIS-NIR transmission spectroscopy and low-resolution proton nuclear magnetic resonance to assess egg HU, but the integration of these spectroscopic techniques did not improve prediction capability when compared with transmission spectral measurements alone. Recently, hyperspectral imaging has been used to determine the internal quality of eggs in terms of HU (Zhang *et al.*, 2015; Suktanarak and Teerachaichayut, 2017), bubble formation, and scattered yolk (Zhang *et al.*, 2015). It appears that the egg HU model

https://doi.org/10.1017/S0043933919000424 Published online by Cambridge University Press

based on hyperspectral imaging performs better than that based on NIR or VIS-NIR spectroscopy (*Table 1*).

In addition to HU, Dong *et al.* (2017a) evaluated albumen pH and whole egg pH using VIS and NIR spectroscopy, reporting correlation coefficients of 0.923 and 0.752, respectively. Fourier transform near-infrared (FT-NIR) spectroscopy was applied for quantitative measurements of thick albumen (R^2 = 0.787; Berardinelli *et al.*, 2005). A more robust model for the thick albumen height was obtained by data pre-processing using a Savitzky-Golay filter and second derivatives (R^2 = 0.873; Hou *et al.*, 2009); this study demonstrated the feasibility of FT-NIR for determining air cell height and air cell diameter (R^2 = 0.895 and R^2 = 0.861, respectively). Kemps *et al.* (2006) confirmed the feasibility of VIS transmission spectroscopy for the non-destructive assessment of egg freshness.

VIS-NIR spectroscopy has been used to determine the HU and pH of the albumen, giving better determination coefficients (Abdel-Nour *et al.*, 2011). Furthermore, both HU and air cell height have been measured by VIS-NIR spectroscopy combined with a special regression method (Aboonajmi and Najafabadi, 2014; Aboonajmi *et al.*, 2016; see *Table 1*). In a study conducted by Giunchi *et al.* (2008), several freshness parameters (air cell height, thick albumen height, and HU) were assessed using FT-NIR (*Table 1*). They found that the HU model was worse than those for the albumen pH, thick albumen height, and air cell height. This could be due to the indirect measurement of HU based on the relationship between the weight of the intact egg and the albumen height and the dependence of the model on the reference measurement.

NIR spectroscopy has been used to assess the storage time of eggs through multivariate analysis methods such as PLS (Abdel-Nour *et al.*, 2011), artificial neural networks (ANNs) (Lin *et al.*, 2011; Coronel-Reyes *et al.*, 2018), and PLS-DA (Giunchi *et al.*, 2008). In fact, PLS-DA models can classify egg samples with perfect accuracy. Fluorescence spectra were evaluated to discriminate eggs according to both their storage time and conditions (Karoui *et al.*, 2008). Regrettably, no fluorescence spectra have since been employed for egg assessments.

Table 1 Application of spectroscopic techniques to the assessment of egg freshness.

Parameter	Spectroscopic technique	Data analysis method	Accuracy	Reference
HU	VIS	MSC, PLS	r=0.842	Kemps *et al.*, 2007
	VIS-NIR	1-Der, PCA-RBRN	R^2_1=0.745 R^2_2=0.76	Aboonajmi *et al.*, 2016
		MLLRR	R^2=0.82	Aboonajmi and Najafabadi, 2014
		MSC, MAXR-PLS	R^2=0.79 RPD=2.16	Abdel-Nour *et al.*, 2011
		MSC, PLS	r=0.888 RPD=2.21	Kemps *et al.*, 2007
	NIR	SNV, GA-SiPLS	R=0.8442 RMSEP=3.29	Lin *et al.*, 2015
		SNV, ICA-GA-ANN	R_P=0.879 RMSEP=2.443	Lin *et al.*, 2011
		SNV, SVDD	93.3%	Zhao *et al.*, 2010
		PLS	R^2=0.676 RPD=1.8	Giunchi *et al.*, 2008
	Hyperspectral imaging	SNV, PLSR	R^2_C=0.91 RMSEC=4.58 R^2_P=0.85 RMSEP=6.29`	Suktanarak and Teerachaichayut, 2017
		SPA-SVR	R^2=0.87 RMSE=4.01% RPD=2.8	Zhang *et al.*, 2015

https://doi.org/10.1017/S0043933919000424 Published online by Cambridge University Press

Table 1 Continued

Parameter	Spectroscopic technique	Data analysis method	Accuracy	Reference
Thick albumen height	NIR	2-Der, PLSR PLS PLS	R^2_P=0.873 R^2=0.789 RPD=1.8 R^2_P=0.787 RMSEP=0.758	Hou *et al.*, 2009 Giunchi *et al.*, 2008 Berardinelli *et al.*, 2005
Albumen pH	VIS	MSC, PLS	r=0.867	Kemps *et al.*, 2006
	VIS-NIR	SNV, PLSR MSC, MAXR-PLS	r_p =0.923 RMSE=0.170 R^2=0.90 RPD=3.32	Dong *et al.*, 2017a Abdel-Nour *et al.*, 2011
Whole egg pH	VIS-NIR	SNV, PLSR	r_p=0.752 RMSE=0.265	Dong *et al.*, 2017b
Air cell height	VIS-NIR	1-Der, PCA-RBRN MLLRR	R^2_1=0.835 R^2_2=0.844 R^2=0.86	Aboonajmi *et al.*, 2016 Aboonajmi and Najafabadi, 2014
	NIR PLS	2-Der, PLSR R^2=0.722 RPD=1.9	R^2_P=0.895 Giunchi *et al.*, 2008	Hou *et al.*, 2009
Air cell diameter	NIR	2-Der, PLSR	R^2_P=0.861	Hou *et al.*, 2009
Storage time	VIS	MSC, regression approach	R=0.935	Liu *et al.*, 2007
	VIS-NIR	MSC, MAXR-PLS	R^2=0.89 RPD=4.92	Abdel-Nour *et al.*, 2011
	NIR	3-Der, ANN SNV, ICA-GA-ANN PLS-DA	R^2=0.8319 RMSECV=1.97 91.4% 100%	Coronel-Reyes *et al.*, 2018 Lin *et al.*, 2011 Giunchi *et al.*, 2008
	Fluorescence	PCA-FDA	94.4%	Karoui *et al.*, 2008
Internal bubbles	Hyperspectral imaging	SVC	90.0%	Zhang *et al.*, 2015
Scattered yolk	Hyperspectral imaging	SVC	96.3%	Zhang *et al.*, 2015

BLOOD AND MEAT SPOTS

Blood and meat spots are common defects inside eggs. The traditional method for detecting blood spots in eggs is candling, although this is labour-intensive and has variable accuracy (from 20 to 90%; Galis *et al.*, 2012). PLS-DA over a wavelength range of 500-600 nm was previously adopted to detect blood-spot eggs, achieving a discrimination rate of 96.8% (Usui *et al.*, 2005).

The above studies only considered white-shelled eggs, and blood detection accuracy in

https://doi.org/10.1017/S0043933919000424 Published online by Cambridge University Press

brown-shell eggs is not as high. Usui *et al.* (2006) detected blood in brown-shell eggs with a discrimination accuracy of only 85% by the traditional blood value method. The reason for the difference in accuracy is that protoporphyrin, a brown pigment in the eggshell, has similar optical properties to those of haemoglobin (De Ketelaere *et al.*, 2004). An improved measurement system combining reflection and transmission was found to be superior to transmission-only measurements. However, for brown-shelled eggs, the detection rate remains low (De Ketelaere *et al.*, 2005). To overcome this problem, various methods have been developed to detect blood-spot eggs. For example, techniques using the generalised Mahalanobis distance (Usui *et al.*, 2006), least-squares support vector machines (Xu *et al.*, 2014), binary logistic regression (Chen *et al.*, 2015), and stepwise Bayesian discriminant analysis (Zhu *et al.*, 2017) have achieved accuracy rates of 93.3%, 91.7%, 96.9%, and 95%, respectively. It is worth mentioning the study carried out by Zhu *et al.* (2015), who detected abnormal eggs, including blood-spot and meat-spot eggs, using a fusion of classifiers. The resulting model was superior to those using a single classifier or base classifier.

Application to hatching eggs

In hatching eggs, one of the main challenges is the detection of infertile and dead embryos at an early stage of incubation. Additionally, in recent years, the sexing of chickens *in ovo* has received increasing attention as a substitute for the culling of day-old male chicks.

FERTILITY AND EMBRYO DEVELOPMENT

Hatchery statistics show that 8-9% of all incubated eggs are unfertilised (Das and Evans, 1992). Candling is traditionally used to remove infertile eggs or dead embryos from the incubator (Tian and Ma, 2011), but this is laborious and time-consuming. The results of several spectroscopic techniques applied to the detection of fertility and embryo development are summarised in *Table 2*.

As early as 2002, VIS transmission spectroscopy was applied to detect embryo development in incubated eggs using the ratio of two wavelengths (577 nm and 610 nm) (Bamelis *et al.*, 2002). Islam *et al.* (2017) proposed non-destructive detection models for hatching egg fertility using VIS transmission spectroscopy. It was concluded that satisfactory classification results can only be obtained after 4-5 d of incubation through VIS transmission spectroscopy, which is very close to the candling time (Tian *et al.*, 2011). Zhu *et al.* (2012) applied NIR spectroscopy to detect infertile eggs prior to incubation and reported an accuracy of 91.67%. Furthermore, hyperspectral imaging has been widely applied to hatching eggs (Smith *et al.*, 2005, 2008; Lawrence *et al.*, 2007; Zhang *et al.*, 2015; Zhu *et al.*, 2014; 2015).

For white-shell eggs, Zhu *et al.* (2014; 2015) realised fertility detection at promising rates of 93% and 93.5% based on an SVM method and GA-propagation (GA-BP) network, respectively. The classification accuracy is generally lower for brown-shell eggs (Smith *et al.*, 2005; 2008). Lawrence *et al.* (2007) achieved 100% accuracy at days two and three of incubation in terms of detecting the embryonic development of hatching eggs, but they used only fertile eggs and a small number of samples ($N = 24$).

In addition, an attempt was made to detect embryo vitality (weak embryo), although the recognition was relatively poor at 81% at day three and 92% at day four of incubation (Zhang *et al.*, 2014). Liu and Ngadi (2013) identified non-fertile eggs (infertile and dead embryo eggs) using a k-means clustering algorithm and NIR hyperspectral imaging.

As well as weak and dead embryos, there exist some slow-developing embryos in

https://doi.org/10.1017/S0043933919000424 Published online by Cambridge University Press

hatching eggs. Kemps *et al.* (2010) developed a quantitative model to assess embryo weight inside the egg from five to ten days of incubation, making it possible to match the incubation length to the needs of the eggs. Similarly, to obtain a batch of homogeneous broiler eggs, scientists have predicted the chick hatching time from the day four incubation spectra (Islam *et al.*, 2015).

Table 2 Application of spectroscopic techniques to the detection of fertility and embryo development.

Parameter	Spectroscopic technique	Incubation time	Shell colour	Data analysis method	Accuracy	Reference
Fertile/ infertile	VIS	Day 4	Light brown	PCA-LDA; PCA-SVM	100%; 100% 97%	Islam *et al.*, 2017
		Day 4; Day 5	Brown and white	PCA-K-means clustering Ratio of two wavelengths (577 nm and 610 nm)	100%	Bamelis *et al.*, 2002
	VIS-NIR	Day 1	Light brown	PCA+Fisher	87.18%	Qin *et al.*, 2017
	NIR	Day 0	White	SNV+1-Der+Norris differential filter, PCA-MD	91.67%	Zhu *et al.*, 2012
	Hyperspectral imaging	Day 0	White	Normalise, PCA-SVM	93%	Zhu *et al.*, 2015
			White	MSC+2-Der, PCA-GA-BP	93.5%	Zhu *et al.*, 2014
		Day 0-Day 3	Brown	PCA-MD	71%; 63%; 65%; 83%	Smith *et al.*, 2008
			Brown	MD	95.8%; 91.7%; 100%; 100%	Lawrence *et al.*, 2007
		Day 3	Brown; White	Ratio of two wavelengths (577 nm and 610 nm)	83%; 91%	Smith *et al.*, 2005
Fertile/non-fertile (infertile; dead embryo)	Hyperspectral imaging	Day 0-Day 4	White	PCA-K-means clustering	100%; 78.8%; 74.1%; 81.8%; 84.1%	Liu and Ngadi, 2013
Development/ weakly developed	Hyperspectral imaging	Day 3; Day 4	Green	PCA-LVQNN	81%; 92%	Zhang *et al.*, 2015
Hatching time	VIS	Day 4	Light brown	MSC, PLS	r=0.873 RMSEP=3.41 h	Islam *et al.*, 2015
Embryonic weight	VIS	Day 5-Day 10	Light brown	MSC, PLS	RPD=4.48	Kemps *et al.*, 2009

https://doi.org/10.1017/S0043933919000424 Published online by Cambridge University Press

SEXING OF CHICKENS *IN OVO*

One-day-old male birds in hatcheries are manually sorted by feather or vent sexing (Biederman and Shiffrar, 1987). The ability to recognise and remove male chicks pre-hatching would be a significant step forward in layer hatcheries. Göhler *et al.* (2016) used hyperspectral imaging for gender determination in layer lines with gender-specific down feather colour. Using totally intact hatching eggs, they obtained an accuracy of approximately 97% at 14 d of incubation. However, a study conducted by Close *et al.* (1997) demonstrated that bird embryos are capable of perceiving pain in the second half of gestation (after 10.5 d of incubation). To overcome this problem, NIR Raman spectroscopy (Galli *et al.*, 2016) and fluorescence spectroscopy (Galli *et al.*, 2017) have been applied to *in ovo* sexing based on differences in the composition of embryonic blood at day 3.5 of egg incubation (before the onset of pain perception), with reported accuracy rates of 90% and 93%. Nevertheless, the need for a window in the egg shell will lead to decreased hatching rates (~10%) (Galli *et al.*, 2016). A minimally invasive method was proposed by Galli *et al.* (2018) based on the same spectral technique and algorithm. In their study, the blood circulation in the extraembryonic vessels was accessed without removing the inner shell membrane.

Conclusions and future outlook

This review summarised recent advances in the quality analysis and classification of chicken eggs using several spectroscopic techniques. Hyperspectral imaging has proved to be a powerful technique for evaluating essential features, both in consumption eggs and hatching eggs. Furthermore, NIR Raman and fluorescence spectroscopy exhibit strong potential for gender determination prior to hatching. In the detection of blood-spot eggs or fertile eggs, eggshell colour proved to be a negative factor.

Numerous properties of eggs have been successfully detected by spectroscopic techniques, but there are still some barriers to overcome. First, large and representative samples are needed to obtain more robust calibrations, and simple, low-cost instruments are required to realise the online monitoring of certain properties in chicken eggs. Furthermore, in future studies, the existence or content of certain minerals in eggs may be detectable by spectroscopic techniques based on changes in physical and chemical properties caused by these minerals.

Acknowledgments

This work was supported by the Beijing Innovation Team of Modern Agricultural Industry Technical System (BAIC04-2016) and the China Agriculture Research System (CARS-41).

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