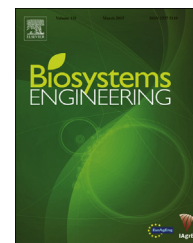


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## Research Paper

# On-line detection of blood spot introduced into brown-shell eggs using visible absorbance spectroscopy

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Blood spots in eggs affect their quality, but it is difficult to realise on-line detection of blood spots in brown-shell eggs because the absorption feature of pigment in brown eggshell is similar to that of the blood spot. The major purpose of this study is to explore the optimal discrimination method based on visible absorbance spectroscopy to realise on-line detection. The spectra of 96 brown-shell normal eggs and 98 brown-shell artificial blood-spot eggs were collected in the spectral range of 200–1100 nm by a prototype egg internal quality detection system with a conveyor speed of 4 eggs per second. Three discrimination methods, partial least squares discriminant analysis (PLS-DA), k-nearest neighbour (KNN) and binary logistic regression (BLR) were used and compared. The results showed that the BLR method was better than PLS-DA and KNN, and the best discrimination rates for the training set and prediction set were 95.4% and 96.9%, respectively. In an external validation with 220 eggs, all three real blood-spot eggs were detected and no normal egg was misjudged by the egg internal quality detection system with BLR model. These results indicated that visible absorbance spectroscopy combined with BLR model could be applied as an on-line detection tool for brown-shell blood-spot eggs.

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## 1. Introduction

Eggs are cheap and rich in nutrition, and have always been an important part of the human diet worldwide (Abdel-Nour, Ngadi, Prasher, & Karimi, 2011). In China, there is a very high domestic consumption at over 300 eggs per capita per year (Ernst, 2009). However some defects like blood spots in eggs will seriously affect the quality of eggs. The pattern of manifestation of blood spot is a red spot or stripe in the egg yolk or in the egg white, and the main reason is due to lack of

vitamins, disease or inheritance (Juliet, 2004). From 2% to 10% of eggs contain blood spots and the existence of blood spots will greatly affect the consumer's feeling and purchase, so it is crucial to select out blood-spot eggs at sale or export (Helbacka & Swanson, 1958; Jensen, Sauter, & Stadelman, 1952). The eggs' grading standard of the United States Department of Agriculture stipulates that the diameter of blood spot in eggs of grade AA and A cannot exceed 1/8 inch (about 3.2 mm). Also, the Chinese Ministry of Commerce stipulates that qualified eggs cannot contain blood spots or other foreign matter. Candling is a traditional method for blood spots

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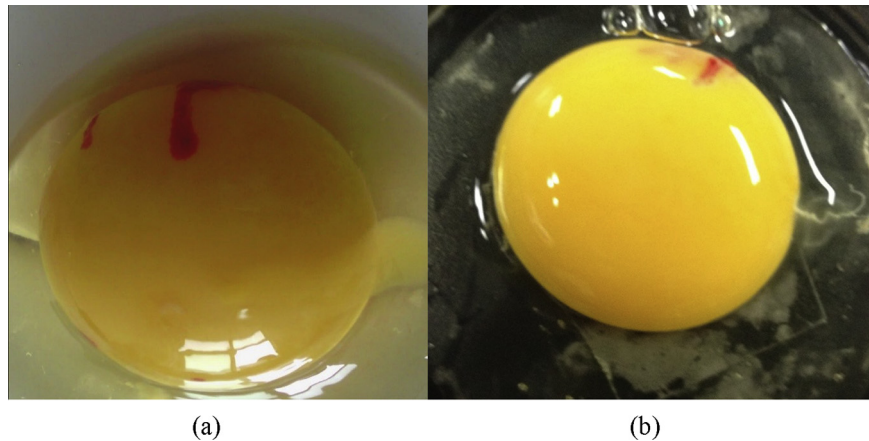


Fig. 1 – (a) a real blood-spot egg, (b) an artificial blood-spot egg.

detection in eggs. However, this method is labour-intensive, and the accuracy varies from 20 % to 90 % (Galiş, Dale, Boudry, & Théwis, 2012).

In the last ten years, visible and near-infrared (Vis/NIR) spectroscopy has become a very popular technique for a wide range of analysis in various industries. The usefulness of this technique is mainly attributed to its allowing the rapid and non-destructive analysis of bulk materials (Bokobza, 2002). For the detection of egg quality, Vis/NIR spectroscopy is often applied to evaluate albumen freshness, including the height of the air space, the pH of the albumen and the Haugh unit (Giunchi, Berardinelli, Ragni, Fabbri, & Silaghi, 2008; Lin, Zhao, Sun, Chen, & Zhou, 2011; Zhao et al., 2010). Using a spectroscopic technique to detect blood spot in eggs was first proposed by Brant, Norris, and Chin (1953). They found that blood has three absorption peaks near 415 nm, 541 nm and 575 nm. After that, Gielen, De Jong, and Kerkvliet (1979) proposed a method for discriminating normal eggs and blood-spot eggs with white shells by using the ratio of absorption peaks at 577 nm and 598 nm (blood value). Usui, Nakano, and Mizutani (2005) detected 100 white-shell normal eggs and 94 white-shell blood-spot eggs by using Vis/NIR spectrum. Partial least squares discriminant analysis (PLS-DA) with a wavelength range of 500–600 nm was adopted, and the discrimination rates for normal eggs and blood-spot eggs were 100% and 96.8%, respectively. Karoui et al. (2009) stated that a small amount of blood in the albumen could only be detected when part of it was diffused in the albumen. The above researches were all for white-shell eggs, while research on brown-shell eggs has been limited. The detection rate of blood spots in eggs is highly dependent on shell colour: for white-shell eggs high detection accuracy can be achieved, while this is not the case for brown-shell eggs (De Ketelaere, Bamelis, Kemps, Decuyper, & De Baerdemaeker, 2004). In fact, the optical properties of protoporphyrin (a brown pigment in the eggshell) are very similar to the haemoglobin (a main component of blood). The protoporphyrin has an adsorption peak at 589 nm which is very close to the absorption peak of haemoglobin (577 nm) which makes the detection of blood in brown-shell eggs very difficult. When Usui, Nakano, and Saitou (2006) detected blood in brown-shell eggs by using traditional blood value discriminant method, the

discrimination rate for blood-spot eggs was just 85%. However, consumers in some markets throughout the world prefer brown-shell eggs to white-shell eggs (Odabaşı, Miles, Balaban, & Portier, 2007).

The purposes of this paper are to use our designed egg quality detection system based on Vis/NIR spectroscopy to analyse the optical properties of brown-shell eggs with or without blood spots, to explore the optimal discrimination model, and finally to realise on-line detection of blood spots in brown-shell eggs.

## 2. Material and methods

### 2.1. Spectral collection system

The egg quality detection system was designed by our team (Xu, Ying, Rao, Wang, & Jiang, 2009). This system is equipped with a roller conveyor with a speed of 0.2 m s<sup>-1</sup> (about 4 eggs per second), a Philips 50 W halogen lamp with a plano-convex lens was set above the conveyor, a low-cost miniaturised Vis/NIR spectrometer (Maya 2000 Pro, Ocean Optics, Inc., USA) was used to collect the transmittance spectra in the range of 200–1100 nm through an optical fibre which was connected to a collimator lens under the conveyor. The integration time for spectral collection was 30 ms and 3 scans per spectrum were averaged to improve signal to noise ratio. Spectral data were recorded as absorbance units by  $\log(1/T)$  where  $T$  is the percent transmittance.

### 2.2. Samples

A total of 194 eggs with brown shell produced in Hubei Jingjiang Egg Industry Co., Ltd were used in this experiment. It is important that the number of test samples should be large enough for spectral calibration and validation analysis, and it is impossible to collect large number of blood-spot eggs at the same time, even in grading and packaging centres, so artificial blood-spot eggs were used. A total of 98 artificial blood-spot eggs were created by injecting 0.05 ml fresh chicken blood for each egg. Figure 1 shows a broken real blood-spot egg and a broken artificial blood-spot egg. From Fig. 1, it can be seen that

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