Feasibility of Detecting Pork Adulteration in Halal Meatballs Using near Infrared Spectroscopy (NIR)

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ABSTRACT

Halal food is widely accepted, with demand increasing yearly. However, due to advancements in food technology, pork adulteration, particularly in homogenous meat products, occurs frequently through attempts to gain monetary benefits. In this study, we used near infrared spectroscopy (NIR) to detect pork adulteration in meatballs. A long wavelength NIR (1000-2500 nm) was used to create a partial least square (PLS) regression model. The results showed that the model of beef meatballs had an R^2-val of 0.88, a SECV of 3.45% and a bias of 0.14%, respectively. For chicken meatballs, the model had an R^2-val of 0.83, a SECV of 4.18%, and a bias of 0.22%, respectively. Considering the strictness of Halal guidelines, the obtained prediction model had a high SECV (3.45% and 4.18% for beef and chicken meatballs) for the detection of pork adulteration in Halal foods. NIR acquisition of meatballs at 25°C and grilled products provided a good result, but frozen meatballs did not. However, considering several advantages of the technique – speed, reduction in required sample preparation and low cost – NIR proved to be a promising technique for screening pork adulteration in Halal meatballs.

Keywords: Near-infrared spectroscopy, Pork adulteration, Meatballs, Halal foods

INTRODUCTION

Islam is one of the world's largest religions. It has been estimated that, in 2009, there were 1.6 billion Muslims, about 23% of the world population (Pew Research Center, 2013). From the Muslim point of view, the decision to choose one food over another depends on its Halal status. Islamic dietary guidelines permit the consumption of Halal foods. According to these guidelines, Muslims cannot consume the following: pork or pork byproducts, animals that are dead prior to slaughter, animals not slaughtered properly or not slaughtered according to Islamic guidelines in the name of Allah, blood, and blood byproducts.

Halal food is widely accepted, with demand increasingly yearly. Most of the food products available on the market show a certificate of Halal label on the package to attract Muslim consumers. However, due to advancements in food technology, adulteration and fraud, via the addition of cheaper meats such as pork,

occurs frequently through attempts to gain monetary benefits from meat products, particularly homogenous meat products. Hence, Muslims tend to doubt the Halal status of these products. There have been attempts to identify pork adulteration in Halal foods using different techniques, such as differential scanning calorimetry (Coni et al., 1994), gas chromatography (Farag et al., 1983), high performance liquid chromatography (Marikkar et al., 2005), electronic nose (Che Man et al., 2005) and DNA-based methods (Aida et al., 2007). However, these methods are laborious, costly and time consuming, and may not be practical for Halal industries.

Near-infrared spectroscopy (NIR) has been recognized as a promising method for the measurement of constituents in agriculture products (Osborne et al., 1993). The NIR spectra are the light spectra ranging from 700 to 2500 mm, lying between visible light and infrared. The operation of NIR depends on the absorption and emitting properties of chemical compounds. Each chemical has its own identity pattern, like a human fingerprint, involving light absorption with overtone and combination bands of fundamental vibrations. NIR allows both qualitative and quantitative chemical analysis, offering several advantages: less time-consuming, no reagent requirement, no waste produced and low cost.

Meatballs are a popular food in Asian countries such as Indonesia, Malaysia and Thailand. However, as meatballs are homogenous products, they could be adulterated with pork, and therefore Muslims question their Halal status. In this study, the feasibility of detecting pork adulteration by NIR was investigated.

MATERIALS AND METHODS

Meatball preparation and pork adulteration

Meatballs were prepared using fresh beef and chicken meat with 10% cornstarch. Then, other ingredients, such as salt, pepper, baking soda and ice water were mixed together in a mixer (Tiger SKF-B, Osaka, Japan) for 5 minutes to homogenize the ingredients. Finally, the obtained homogenous mixture was shaped by hand. The raw meatballs were immersed in warm water (50°C) for 20 minutes to set the gel. Then, they were cooked in hot water (90°C) for 20 minutes, kept in cold water (5°C) for 15 minutes and dried in open air (10 minutes).

For pork adulteration, 0, 5, 10, 15, 20, 25 and 30 % (w/w) of pork was added to the beef or chicken during the preparation of meatballs. Ten replications of meatballs samples were prepared for each formula.

NIR spectral acquisition

Before NIR acquisition, the beef, chicken and adulterated meatballs were set separately according to the following conditions: Condition 1- the meatball was cut at the middle into two pieces using a sharp knife, and the obtained cut meatball was incubated in a water bath covered with thin polyethylene at 25°C for 30 minutes; Condition 2- the cut meatballs were kept in a freezer for 12 hours and NIR spectra were obtained while the meatballs were in a frozen state; Condition 3- the meatballs were cooked by grilling in an electric oven (1000 watts for 5 minutes). The grilled meatball was cut into two pieces and the cut meatball was

incubated in a water bath at 25°C for 30 minutes before spectral acquisition. To acquire NIR spectra, the smooth surface of the cut meatball was used to contact the NIR acquisition part. The NIR spectra were acquired using a Buchi NIRFlex N-500 instrument (Buchi, Switzerland), equipped with N510-000 NIRFlex Solids, with a reflectance mode at a wavelength of 1000-2500 nm.

Data analysis

The NIR spectra and the value (%) of pork adulteration were imported to Unscrambler software (version 9.8, GAMO, Oslo, Norway) for calculation. The spectral pre-treatment – smoothing, multiplicative scatter correction (MSC) and derivative transformation – were applied to the original spectra. Consequently, the wavelength range was optimized as per the following procedure: the lowest wavelength was fixed and the longer wavelength was changed to obtain the best statistical results (1000-2500, 1000-2300, 1000-2100 and 1000-1900 nm). Further, the shorter wavelength was adjusted to find the best results at the fixed long wavelength from the previous results. The model was created by partial least square (PLS) regression using a "leave-one-out" cross validation procedure. The cross validation process split samples into train and test sets. Each sample was used once as a test set, while the remaining samples were used as a training set to create a model. Statistical criteria – standard error of calibration (*SEC*), standard error of cross-validation (*SECV*) and the coefficient of determination (R^2) – were considered to obtain the best model.

RESULTS

Original spectra of meatballs

The original spectra of beef, chicken and pork meatballs (100% of each meat) are shown in Figure 1A. The differences of NIR absorption covering all wavelengths might be a result of the scattering effect. The water band (1400 nm) and protein bands (1700 nm and 2100 nm) could be observed (Yongni et al., 2011; Fertig et al., 2004). The original spectra of beef and chicken meatballs with different pork adulteration (0, 5, 10, 15, 20, 25 and 30% wt.) are shown in Figures 1B and 1C. The results show that pork adulteration in the meatballs may affect the NIR absorption, particularly at wavelengths of 1400 nm and 1600-2100 nm.

Calibration and prediction

The original spectra of beef and chicken meatballs were treated with spectra pre-treatments and PLS models were created. The original spectra were the best model for beef meatballs, while NIR spectra treated with smoothing were the best results for chicken meatballs. However, considering other statistical criteria for chicken meatballs, the original spectra provided a good result, with a smaller number of factors (*F*) of 13, than those of smoothed spectra of 16, in creating a model. Therefore, in this study, the original spectra were chosen to optimize the wavelength range. The results indicated that the best mode was obtained when using whole wavelength ranges (at 1000-2500 nm) in both beef meatballs and

chicken meatballs (data not shown). The models of meatballs at NIR acquisition (Condition 1 at 25°C) are shown in Table 1.

For beef meatballs, the best model for the detection of pork adulteration was obtained when using a wavelength of 1000-2500 nm. The beef meatball model was calculated from 12 factors, with an R^2-*cal* of 0.96, a *SEC* of 1.99%, an R^2-*val* of 0.88, a *SECV* of 3.45% and a bias of 0.14%. For chicken meatballs, the model was created using 13 factors at 1000-2500 nm, with an R^2-*cal* of 0.96, a *SEC* of 1.78%, an R^2-*val* of 0.83, a *SECV* of 4.18% and a bias of 0.22%.

The regression coefficients and the scatter plot of pork adulteration in beef meatballs are shown in Figure 2. The plot is complicated with the presence of many noisy peaks. This could be due to the more than 10 factors that were used to create the models, resulting from the complexity of the intact meats, such as water, lipid and protein, which have good responses to NIR spectra. The wavelengths at 1395 nm and 1725 nm were linked to combination vibrations of C-H and wavelengths of 1980 nm, 2050 nm, 2180 nm and 2294 nm showed high correlation involving protein vibration bands (Fertig et al., 2004).
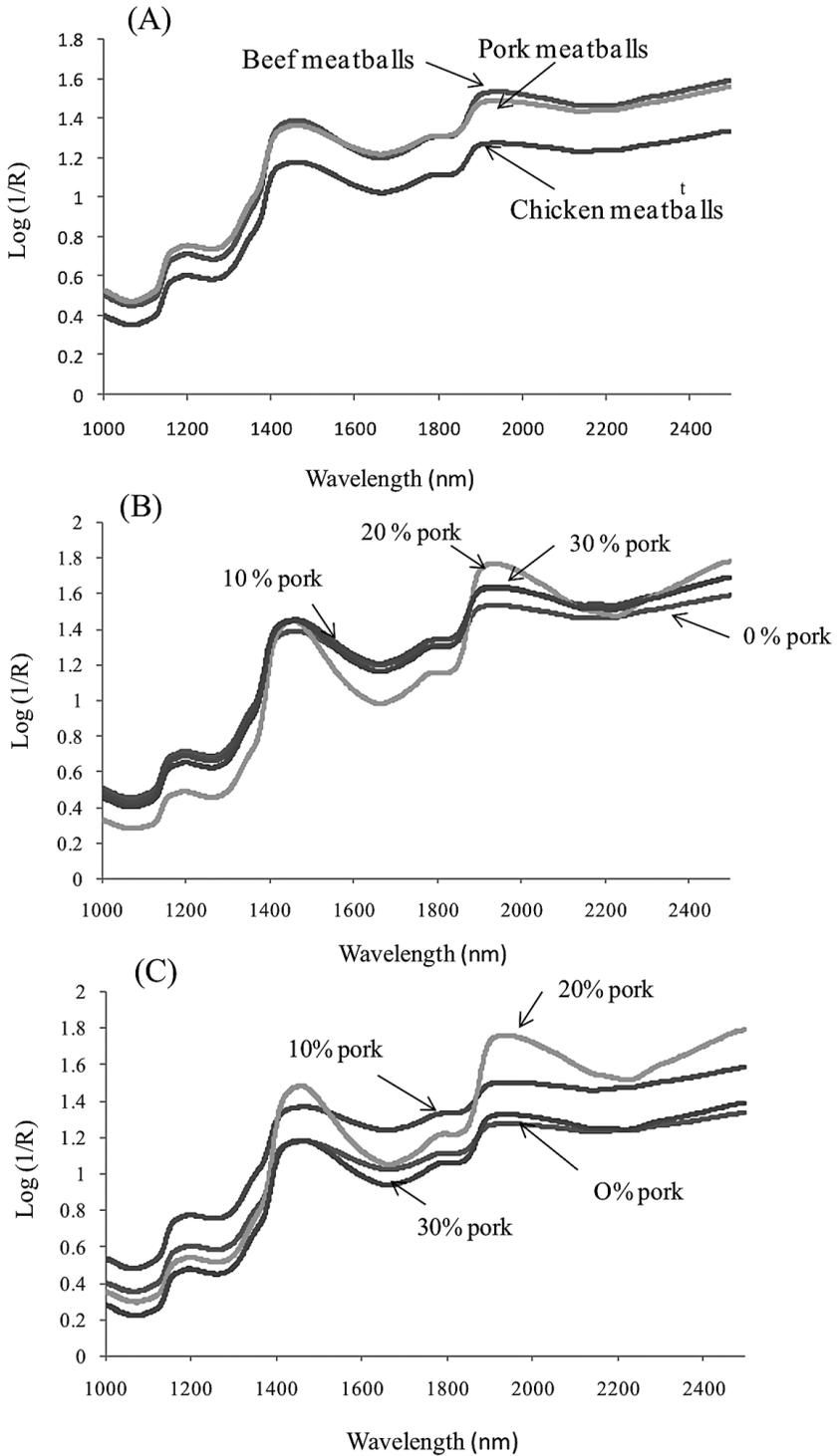
For chicken meatballs, the regression coefficients and the scatter plot of actual pork adulteration are shown in Figure 3. The regression coefficients plot is complicated, similar to the results obtained for beef meatballs. However, the wavelength peaks involving vibrations of C-H (1215 nm, 1725 nm and 2132 nm) and proteins (1530 nm and 2050 nm) were markedly observed (Osborne et al., 1993).

**Effect of acquisition conditions**

The objective of this part is to determine the feasibility of NIR for identifying pork adulteration under practical conditions matching available meatball products on the market. The spectra of the meatballs were acquired at different conditions (Condition 1- spectral acquisition at 25°C, Condition 2- frozen products and Condition 3- grilled meatballs). The regression models of different conditions are shown in Table 2.

The beef meatballs acquired at Condition 1 (25°C) and Condition 3 (grilled meatballs) had almost identical results, with an R^2-*val* of 0.88 and a *SECV* of 3.45%, and an R^2-*val* of 0.86 and a *SECV* of 3.75%, respectively. The frozen meatballs (Condition 2) showed less of an R^2-*val* (0.44) with a high *SECV* (7.57%). Similarly, the results of chicken meatballs set at Condition 1 and Condition 3 showed good results in predicting pork adulteration, with *SECV* of 4.18% and 4.49%, respectively. However, for frozen meatballs, the result was not satisfactory, with an *SECV* of 9.30%.
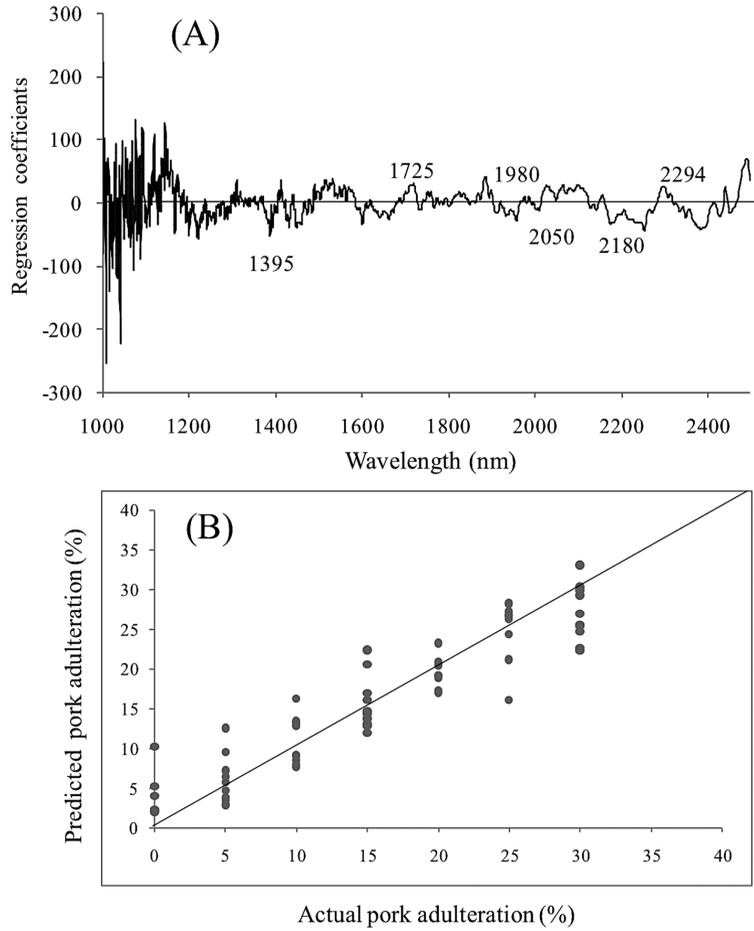
The regression coefficient plots of different conditions of beef meatballs are shown in Figure 4. For meatball spectra at 25°C and grilled meatballs, high correlations at the same wavelength are observed at 1530 nm, 2040-2050 nm, 2180 nm and 2310 nm. For frozen meatballs, a high relationship at 1400 nm, 1890 nm and 1940 nm can be observed and linked to the vibration of the water bands (Osborne et al., 1993).

Figure 1. The original spectra of beef, chicken and pork meatballs (A) the original spectra of beef meatballs with pork adulteration (B), and original spectra of chicken meatballs with pork adulteration (C).
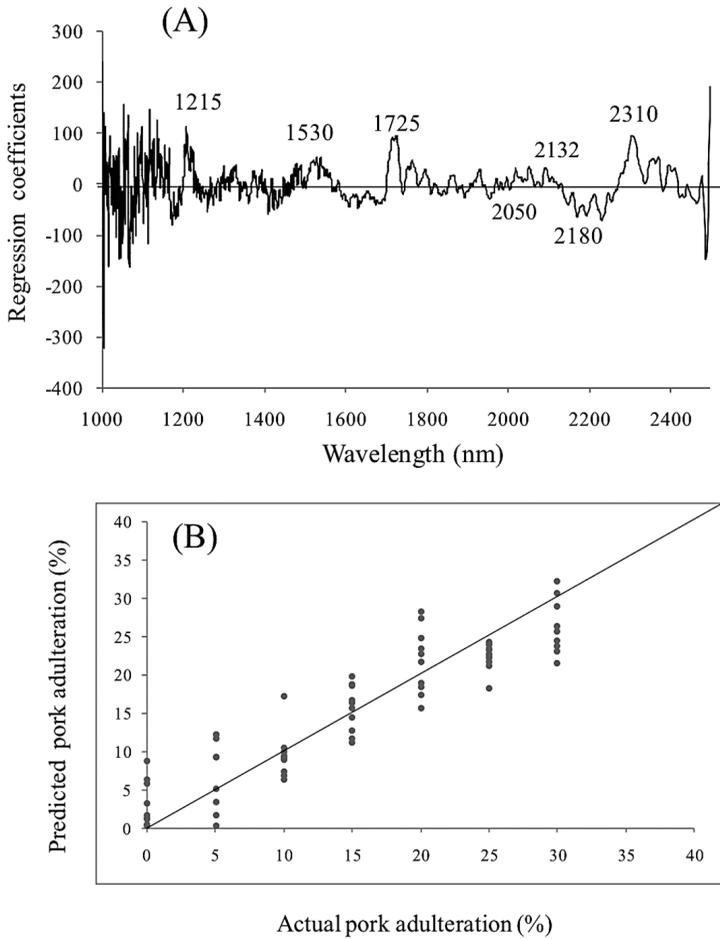
Figure 2. The regression coefficients (A) and scatter plot of actual vs. computed pork adulteration in beef meatballs (B).

Figure 3. The regression coefficients plot (A) and scatter plot of actual vs. computed pork adulteration (B) of chicken meatballs.
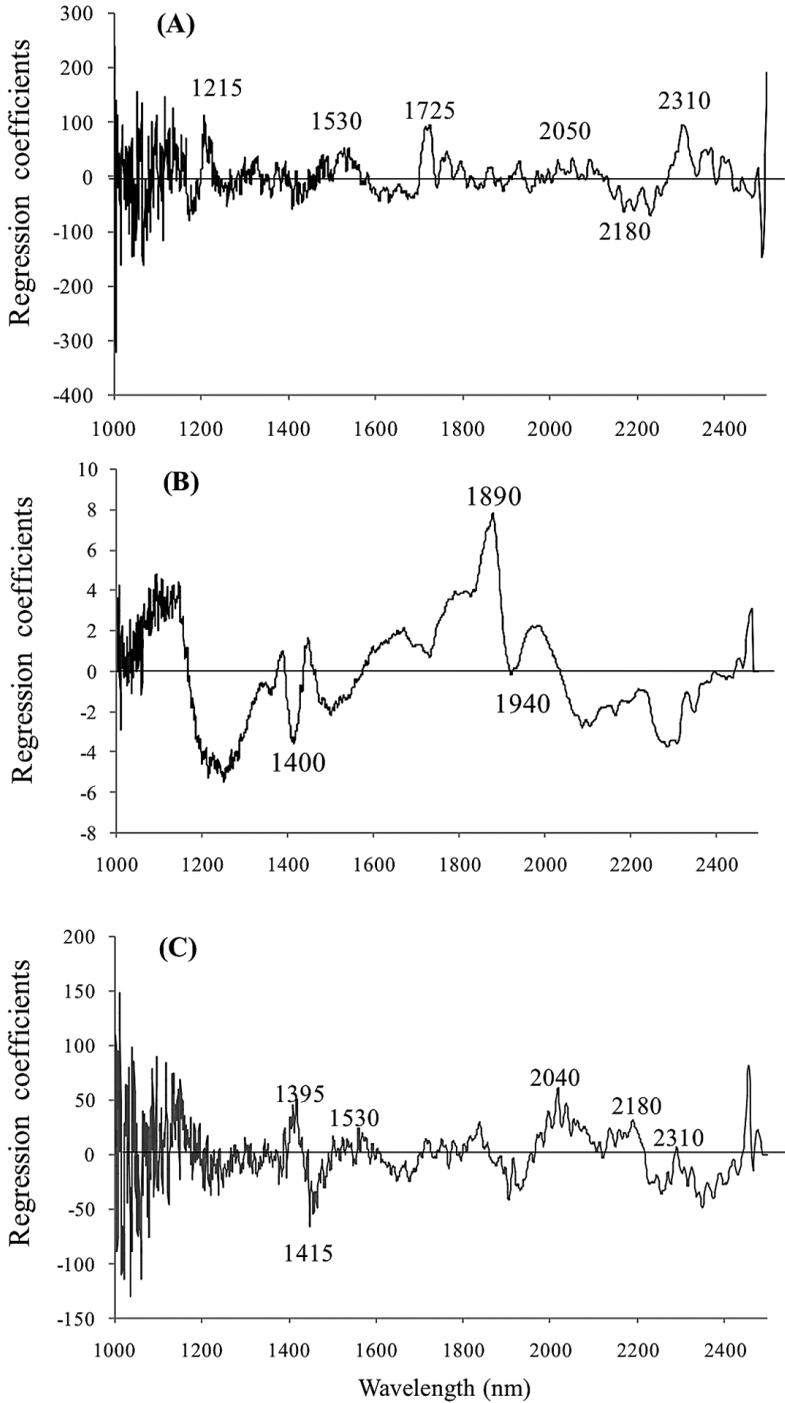
Figure 4. The regression coefficient plots of the beef meatballs acquired at condition 1 (at 25°C; A), condition 2 (frozen meatballs; B) and condition 3 (grilled meatballs; C).
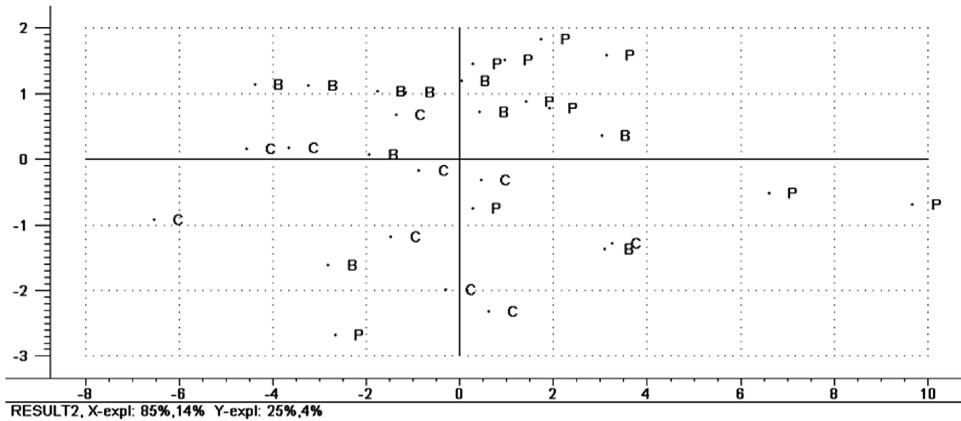
Figure 5. The score plot of beef (B), chicken (C) and adulterated (P) meatballs.

Table 1. PLS calibration and validation results for pork adulteration (0-30%) in beef and chicken meatballs.

Meatball	Treatment	Wavelength (nm)	F	R^2-cal	SEC	R^2-val	SECV	Bias
Beefball	Original	1000-2500	12	0.96	1.99	0.88	3.45	0.14
Beefball	Smoothing(13)	1000-2500	13	0.95	2.03	0.87	3.62	0.07
Beefball	2nd derivative	1000-2500	4	0.95	2.06	0.84	3.99	-0.01
Beefball	MSC	1000-2500	11	0.96	1.82	0.85	3.82	0.24
Chick-ball	Original	1000-2500	13	0.96	1.78	0.83	4.18	0.22
Chick-ball	Smoothing(13)	1000-2500	16	0.98	1.05	0.83	4.11	0.03
Chick-ball	2nd derivative	1000-2500	5	0.97	1.49	0.80	4.57	-0.03
Chick-ball	MSC	1000-2500	11	0.95	2.06	0.82	4.37	0.31

Note: *F*: number of factors; R^2: the coefficient of determination; *SEC*: standard error of calibration, *SECV*: standard error of cross-validation; Bias: the average of differences between reference value and NIR value.

Table 2. PLS calibration and validation results for pork adulteration (0-30%) in beef and chicken meatballs in different conditions (calculation with original spectra).

Meatball	Condition	Wavelength (nm)	F	R^2-cal	SEC	R^2-val	SECV	Bias
Beefball	25°C	1000-2500	12	0.96	1.99	0.88	3.45	0.14
Beefball	Frozen	1000-2500	11	0.74	5.13	0.44	7.57	-0.27
Beefball	Grill	1000-2500	14	0.98	1.38	0.86	3.75	-0.05
Chick-ball	25°C	1000-2500	13	0.96	1.78	0.83	4.18	0.22
Chick-ball	Frozen	1000-2500	7	0.39	7.86	0.15	9.30	0.25
Chick-ball	Grill	1000-2500	15	0.98	1.31	0.80	4.49	0.06

Note: *F*: number of factors; R^2: the coefficient of determination; *SEC*: standard error of calibration, *SECV*: standard error of cross-validation; Bias: the average of differences between reference value and NIR value.

DISCUSSION AND CONCLUSION

According to Halal food guidelines, meat from pigs is strictly prohibited. In this study, we investigated the feasibility of detecting pork adulteration by NIR. For intact fresh meats, beef, chicken and pork showed notable differences in color, viscosity and density (data not shown). However, when mixing each meat together, it is quite difficult to detect and separate. To understand this matter, a score plot of meatballs with no pork adulteration and adulterated meatballs was created (Figure 5). The score plot expressed that the meatballs could not be grouped and a scattering distribution among the meatballs could be observed. This can be explained by each meat containing some of the same compounds, such as amino acid, protein, fat and water. As per the results in this study, the wavelengths linking to those compounds could be notably observed in the regression coefficients plots of all samples (beef, chicken and pork adulterated meatballs). This is due to each meat containing different amounts of these chemical compounds and being sensitively affected by NIR spectra, resulting in complexity of NIR absorption. Due to this reason, the obtained pork adulterated models had a high *SECV* with a low accuracy of prediction. Noguchi et al. (2012) reported that it is difficult to classify the minced meats of beef, pork and chicken using chemical compositions (moisture, crude fat, protein and fatty acid) by NIR when the meats are mixed together, because the chemical compositions are widely distributed, depending on the portions of each meat.

The NIR acquisition of the meatballs at 25°C and as grilled products provided a good result, but frozen meatballs did not. This can be explained by the status of the water in frozen products changing from water (liquid) into ice (solid) inside the matrix of meatballs with disordered distribution. This affected NIR spectra absorption in long wavelengths (1000-2500 nm) and through the scattering properties of light by ice molecules. Therefore, a poor model for frozen meatballs was obtained.

Considering strict Halal guidelines, the error of pork adulteration in meatballs was quite high (3.45% SECV for beef and 4.18% SECV for chicken meatballs) and is not acceptable for Muslims. However, considering the numerous advantages, such as speed, reduction in sample preparation required and low cost, NIR could be used as an initial screening step for identification of pork adulteration in meatballs via rapid analysis.

In conclusion, we used near infrared spectroscopy (NIR) to detect pork adulteration in meatballs. The best model was obtained when a wavelength of 1000-2500 nm was used. For beef meatballs, the model had an R^2-*cal* of 0.96, a *SEC* of 1.99%, an R^2-*val* of 0.88, a SECV of 3.45% and a bias of 0.14%. For chicken meatballs, the model had an R^2-*cal* of 0.96, a *SEC* of 1.78%, an R^2-*val* of 0.83, a *SECV* of 4.18% and a bias of 0.22%. NIR acquisition of the meatballs at 25°C and as grilled products provided a good result, but frozen meatballs did not. Considering the need for strictness of Halal food guidelines, the models had quite a *SECV* for use in determining Halal status. However, this model could be applied as an initial screening step for the detection of pork adulteration.

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