

# COMPARISON OF VIS-NIR (400-1,000 NM) AND NIR (978-1,678 NM) HYPERSPECTRAL IMAGING FOR DISCRIMINATION BETWEEN FRESH AND PREVIOUSLY FROZEN POULTRY

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## ABSTRACT

The objective of this study is to compare hyperspectral imaging (HSI) techniques in the Vis-NIR (400-1000 nm) and NIR (978 – 1,678 nm) regions to discriminate between fresh and previously frozen poultry with and without skin. Partial least squares discriminant analysis (PLS-DA) models were built at the pixel and object level using data pre-treated with multiplicative scatter correction (MSC). Successful discrimination between fresh and previously frozen poultry could be accomplished using pixel level models built on Vis-NIR data. The best PLS-DA results were achieved by using samples with skin present, resulting in a correct classification of 84.2% in the training set and 88.2% in the test set (LV = 10), sensitivity of 92.1% and specificity of 84.2%. Wavelengths determined to be important for discrimination by the regression coefficients of the PLS-DA model include: 500, 575, 595, and 620 nm. These wavelengths are associated with myoglobin and its derivatives, which are mainly responsible for the colour of meat. Discrimination of the two classes in the NIR range was poor in samples with and without skin. No object level classification models were successful.

**Index Terms**— Hyperspectral imaging, poultry, fresh, frozen-thawed, PLS-DA

## 1. INTRODUCTION

To prevent fresh poultry from perishing, retailers may choose to freeze meat to prolong shelf life of products. However, freezing reduces product quality and is therefore perceived as undesirable by consumers in comparison with fresh meat. Specifically, lipid and protein oxidation increases while water holding capacity decreases and colour changes with each freeze-thaw cycle [1]. In an attempt to meet consumer demands for fresh meat while benefiting from increased shelf life of frozen meat, retailers may be influenced to label products thawed from frozen as fresh meat. To ensure product quality, authentication techniques are required to ensure correct labelling. Currently used authentication techniques include enzymatic, DNA based, spectroscopic, bio-imaging, and sensory analytical methods. Although current non-spectroscopic methods are capable of discriminating between fresh and previously frozen meat, they are time consuming, destructive, and rely on trained professionals. Novel techniques, such as hyperspectral imaging (HSI), are being developed to acquire real-time chemical and spatial

information about products without destruction of samples to ensure quality of products and prevent economic losses. Different HSI systems operate by imaging in different spectral ranges. Although previous point spectroscopic studies have made successful discrimination between fresh and frozen-thawed chicken breasts in the wavelength range of 400 – 2498 nm [2], HSI has not yet been applied to discrimination between fresh and thawed poultry.

## 2. METHODS AND MATERIALS

### 2.1. Poultry samples

Fresh chicken breast fillets ( $n_{\text{samples}} = 18$ ) were acquired from a local supermarket. All samples were from free range, grain fed chickens, traceable to the same farm. Individual samples were transferred from their original modified atmosphere packaging (MAP) into polyethylene-linear low density (LLDPE) freezer bags. Samples were randomly assigned to control ( $n_{\text{samples}} = 9$ ) and treatment ( $n_{\text{samples}} = 9$ ) groups. Control samples were kept at 4°C for the duration of the experiment. Treatment samples were frozen at -15°C for 24 h, after which they were moved to 4°C for a further 24 h. Samples were dried using a paper towel and then imaged on the ventral side in the NIR wavelength range. Next, the entire procedure was repeated on a separate day with a new set of samples and imaged on the ventral side in the Vis-NIR wavelength range. The protocol was then repeated using chicken thighs with skin on ( $n_{\text{samples}} = 18$ ).

### 2.2. Hyperspectral imaging system

The Vis-NIR HSI system used operated from 400 – 1,000 nm with a spectral resolution of 5 nm. Images were 580 rows x 775 columns x 121 bands. The NIR HSI system used operated from 880 - 1720 nm with a spectral resolution of 7 nm. Images were 320 rows x 580 columns x 121 bands. All samples were paced on a white tile for imaging.

### 2.3. Software

Hyperspectral images were obtained using the acquisition software SSanner (v1.4.5, DV Optics, Padua, Italy). All data analysis was completed using MATLAB R2018b (MathWorks, Massachusetts, USA).

### 2.4. Image segmentation

Vis-NIR images were segmented using Otsu's method [3] for automatic threshold selection to partition pixels on greyscale

images at 440 nm. A closing morphological operation was used to dilate the mask and include all pixels of interest.

In order to reduce noise in NIR images, the spectral range was reduced to 978 - 1678 nm. Greyscale images at 1503 nm were used to segment samples from background, using Otsu's method [3] for automatic threshold selection to partition pixels.

## 2.6. Data analysis

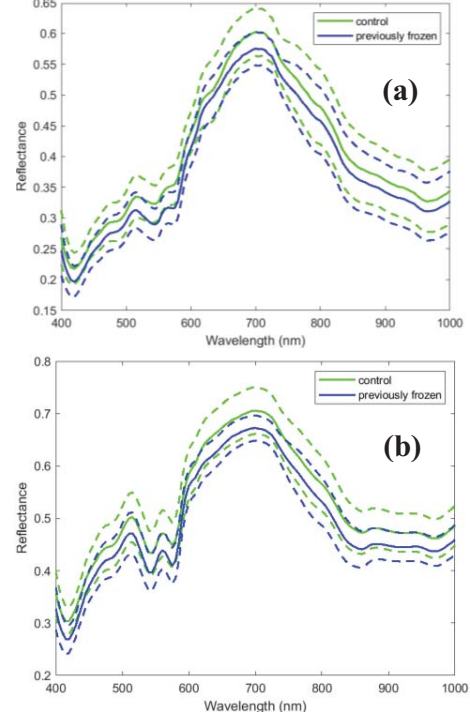
For both Vis-NIR and NIR sets of images, samples were split into a training ( $n_{\text{samples}} = 12$ ) and independent test set ( $n_{\text{samples}} = 6$ ) to be used for building and evaluating partial least squares discriminant analysis (PLS-DA) models. Models were built on spectra pre-treated with multiplicative scatter correction (MSC). Pre-treated mean spectra of whole samples were used to build object level models. Models were compared based on resultant correct classification (%) of the independent test set, sensitivity and specificity.

## 3. RESULTS AND DISCUSSION

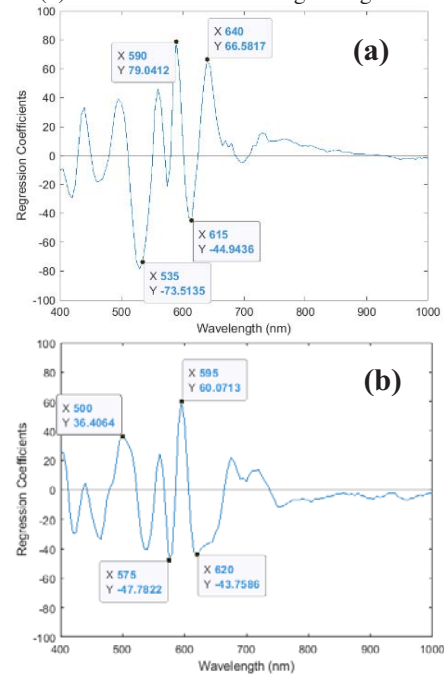
### 3.1. Vis-NIR (400-1000 nm)

Mean reflectance spectra of fresh and previously frozen poultry samples are most noticeably visually different between the wavelength range of 400-600 nm (Figure 1). Models to discriminate between fresh and previously frozen chicken breast fillets using HSI performed favourably only in the Vis-NIR range, as shown in Table 1. The best PLS-DA model for samples without skin was achieved at the pixel level, resulting in a correct classification of 89.8% in the training set and 81.7% in the test set ( $LV = 10$ ), sensitivity of 77.6% and specificity of 90.8%. Wavelengths determined to be important for discrimination by the regression coefficients of the PLS-DA model include: 535, 590, 615, and 640 nm (Figure 2). For samples without skin, the best model was achieved at the pixel level, resulting in a correct classification of 84.2% in the training set and 88.2% in the test set ( $LV = 10$ ), sensitivity of 92.1% and specificity of 84.2%. Wavelengths determined to be important for discrimination by the regression coefficients of the PLS-DA model include: 500, 575, 595, and 620 nm (Figure 2). Wavelengths determined to be important for discrimination are associated with myoglobin and its derivatives, which are mainly responsible for the colour of meat [4]. Freezing of meat changes the spectrum of myoglobin and its derivatives, resulting in a colour change in previously frozen meat. However, this colour change is not perceptible by the human eye. Spatially, misclassified pixels are observed to occur mainly on the outer edges of samples (Figure 3). No models were successful at the object level. For samples without skin, correct classification of 50.0% in the training set and 66.7% in the test set ( $LVs = 5$ ), sensitivity of 60.0% and specificity of 100%. For samples without skin, correct classification of

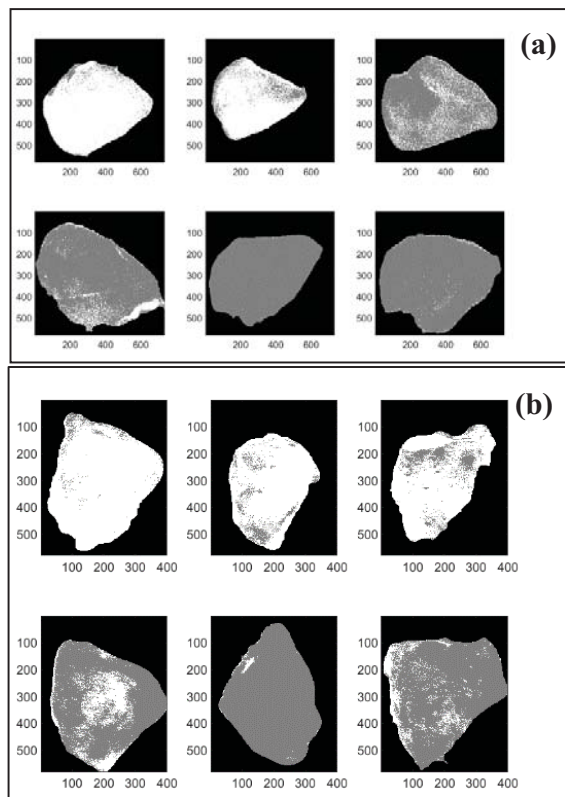
50% in the training set and 66.7% in the test set ( $LVs = 4$ ), sensitivity of 55.0% and specificity of 61.4%.



**Figure 1.** Mean reflectance ( $\pm 1$  SD) spectra of fresh ( $n_{\text{samples}} = 9$ ) and previously frozen ( $n_{\text{samples}} = 9$ ) poultry samples without skin (a) and with skin (b) in the Vis-NIR wavelength range.



**Figure 2.** Regression coefficients from PLS-DA model achieved by using samples without skin (a) and with skin (b) in the Vis-NIR wavelength range.

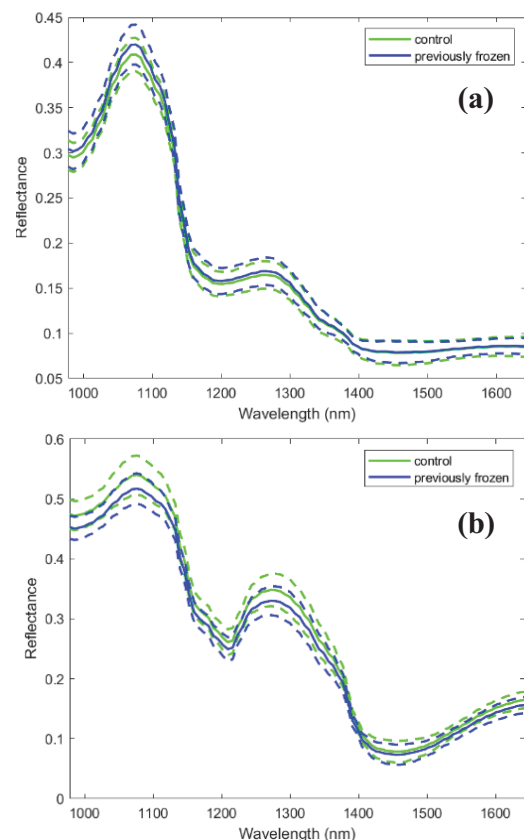


**Figure 3.** PLS-DA model achieved by using data pre-treated with MSC applied to hyperspectral images of samples without skin (a) and with skin (b) in the Vis-NIR wavelength range. The top row of each figure shows the fresh samples, and the bottom row shows previously frozen samples. White represents pixels classified as fresh and grey pixels represent pixels classified as previously frozen.

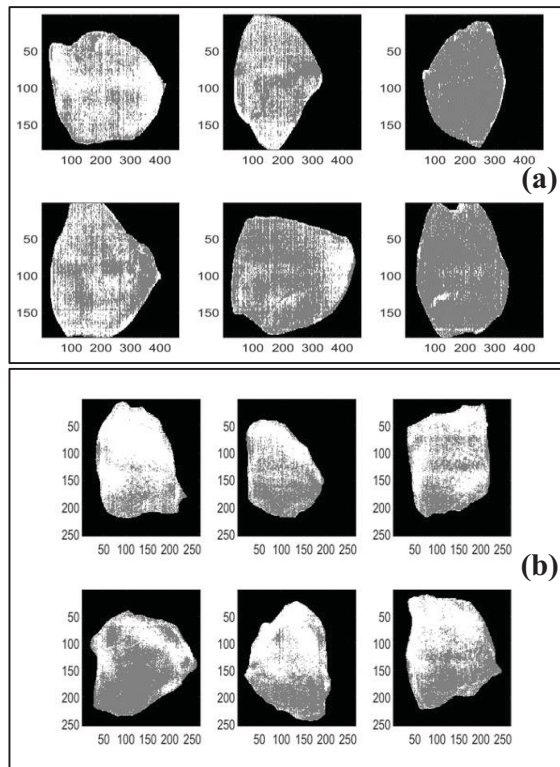
### 3.2. NIR (978 – 1,678 nm)

Mean reflectance spectra of fresh and previously frozen poultry samples are not noticeably visually different (Figure 4). Discrimination of the two classes in the NIR range was poor in samples with and without skin at both pixel and object level. The best PLS-DA model for samples without skin resulted in a correct classification of 65.7% in the training set and 59.1% in the test set (LV = 12), sensitivity of 55.0% and specificity of 61.4%. The best PLS-DA model for samples without skin resulted in a correct classification of 66.2% in the training set and 56.8% in the test set (LV = 10), sensitivity of 61.0% and specificity of 53.5%. For samples without skin at the object level, correct classification of 50.0% in the training set and 66.7% in the test set (LVs = 9), sensitivity of 60.0% and specificity of 100%. For samples without skin, correct classification of 50% in the training set and 66.7% in the test set (LVs = 5), sensitivity of 66.7% and specificity of 66.7%. Spatially, misclassified pixels are observed to occur everywhere in the samples (Figure 5). Poor discrimination

was likely a result of increased absorbance of surface moisture due to drip loss from decreased water holding capacity of samples during storage throughout the duration of the experiment [5]. Previous spectroscopic work was able to discriminate between fresh and previously frozen poultry using bands in the 1400-1600 nm region [2]. These distinctive bands are a result of overlapping first overtones of OH/NH stretching modes of water interacting with itself and with protein complexes, typical of meat composition [2]. Samples used in the previous work were packaged 8 h post-mortem and immediately placed into treatment temperature [2]. In contrast, samples used for this study were stored for an unknown amount of time prior to the experiment in MAP packaging. Rather than receiving the signals of the OH/NH stretching modes expected from previous work, the system may have been limited by the strong absorbance water due to increased surface moisture from drip loss.



**Figure 4.** Mean reflectance ( $\pm 1$  SD) spectra of fresh ( $n_{\text{samples}} = 9$ ) and previously frozen ( $n_{\text{samples}} = 9$ ) poultry samples without skin (a) and with skin (b) in the NIR wavelength range.



**Figure 5.** PLS-DA model achieved by using data pre-treated with MSC applied to hyperspectral images of samples without skin (a)

and with skin (b) in the NIR wavelength range. The top row of each figure shows the fresh samples, and the bottom row shows previously frozen samples. White represents pixels classified as fresh and grey pixels represent pixels classified as previously frozen.

#### 4. CONCLUSION

Successful discrimination between fresh and previously frozen poultry could be accomplished using HSI in the Vis-NIR (400-1,000 nm) wavelength range at the pixel level. Discrimination was most successful on samples with skin present. However, it was not possible to successfully discriminate between fresh and previously frozen poultry using HSI in the NIR (978-1,678 nm). Poor discrimination in the NIR region was likely a result of increased absorbance of surface moisture due to drip loss from decreased water holding capacity of samples during storage throughout the duration of the experiment. No models were successful at the object level. Future work could extract data at wavelengths of importance to eliminate redundant information for discrimination and could improve classification results by modifying masking techniques to exclude edges of samples.

#### 5. ACKNOWLEDGEMENTS

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**Table 1.** Correct classification results (%) of PLS-DA models and number of latent variables (LVs) used on fresh ( $n_{\text{samples}} = 9$ ) and previously frozen ( $n_{\text{samples}} = 9$ ) poultry samples with and without skin in the Vis-NIR and NIR wavelength range.

Skin	Level	Vis-NIR (400-1,000 nm)					NIR (978 – 1,678 nm)				
		Correct classification (%)		Sensitivity (%)	Specificity (%)	LVs	Correct classification (%)		Sensitivity (%)	Specificity (%)	LVs
		Training (n)	Test (n)				Training (n)	Test (n)			
Absent	Pixel	89.8 (2189797)	81.7 (117921)	77.6	90.8	10	65.7 (523162)	59.1 (262955)	55.0	61.4	12
	Object	50.0 (12)	66.7 (6)	60.0	100.0	5	50.0 (12)	66.7 (6)	60.0	100.0	9
Present	Pixel	84.2 (1370176)	88.2 (665714)	92.1	84.2	10	66.2 (373968)	56.8 (184784)	61.0	53.5	10
	Object	50.0 (12)	66.7 (6)	60.0	100.0	4	50.0 (12)	66.7 (6)	66.7	66.7	5

## 6. REFERENCES

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