# Selection of visible/near infrared wavelengths for characterising fecal and ingesta contamination of poultry carcasses

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## Introduction

Zero tolerance of feces on the surfaces of animal carcasses during slaughter was established as a standard by the Food Safety Inspection Service (FSIS) to minimise the likelihood of contamination of meat and poultry with microbial pathogens.[1] Compliance with zero tolerance in meat processing establishments is currently verified by visual observation. Three criteria are used for identifying fecal contamination. These are colour, consistency and composition. Inspectors use these guidelines to verify that establishments prevent carcasses with visible fecal contamination from entering the immersion ice water bath (chiller). Real-time visual inspection is both labour-intensive and prone to both human error and variability.

Efforts have been made to develop automated or semi-automated visual inspection systems for detecting the presence of contaminants on food products during processing. These systems utilise a technique in which the food item is illuminated with UV or visible light and emissions of fluorescent light is measured between 660 to 680 nm as an indication of the presence of fecal material.[2,3] Visible and near infrared (vis/NIR) reflectance spectroscopy is a technique that can be used to detect contamination on foodstuffs. Vis/NIR spectroscopic techniques have been used to classify wholesome, speticemic and cadaver carcasses.[4,5] The vis/NIR method showed promise for separation of wholesome and unwholesome carcasses in a partially automated system.

Multispectral and hyperspectral imaging systems also have the potential for inspection of meat products during processing. Hyperspectral imaging has been used for the identification of surface contaminates on poultry carcasses.[6,7] Hyperspectal and multispectral imaging techniques were used to detect chicken skin tumors.[8] Park *et al* reported a hyperpspectral imaging system for detecting feces and ingesta on the surfaces of poultry carcasses.[9] The objectives of this research are to investigate the use of visible/ NIR reflectance spectroscopy to discriminate between uncontaminated poultry breast skin and pure feces and to select optimum or key wavelengths.

## Materials and methods

### Broilers and processing procedures

Male broilers were obtained from a local commercial farm at 38 days of age and transported to grow-out facilities. Broilers were provided a non-medicated, corn-soybean meal pelleted growers diet (3,200 kcal ME $kg^{-1}$, 19% crude protein) *ad libitum*. Feed and water were withdrawn eight hours and



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four hours prior to processing the birds, respectively. In Experiment 1, two replicates with 20 broilers were stunned, bled in shackles for a total of 120 sec, scalded at 57°C (hard scald) for 2 min and defeathered in the Russell Research Center pilot scale processing facility. In Experiment 2, two replicates of 16 broilers were scalded at 57°C and 52°C (soft scald). To collect intestinal content, broilers were eviscerated to obtain fecal material from the duodenum, ceca and colon portion of the viscera. Ingesta was collected from the proventriculus and/or gizzard. Fecal and ingesta contents were pressed from the dissected viscera into sample vials for analysis by visible/NIR spectroscopy. Circular samples of skin (38 mm diameter) were taken from the breast area of the carcass and placed in plastic bags.

### Spectroscopic and multivariate analysis

Fecal, ingesta and breast skin samples were scanned with an NIRSystems 6500 monochromator (NIRSystems, Silver Spring, MD, USA). Spectra were recorded from 400 nm to 2500 nm at 2 nm intervals and analysed from 400 nm to 950 nm to correspond with the wavelength range of the hyperspectral imaging system described by Park *et al*.[9] Three replicates of uncontaminated breast skin from each carcass were presented in cylindrical sample cells. Samples of pure feces were presented in cylindrical sample cells (internal diameter: 38 mm; depth: 0.1, 0.2, or 0.3 mm) with an optical quartz surface and a locking back.

A commercial spectral analysis program (NIRS3, Infrasoft International, Inc., Port Matilda, PA, USA) was used to analyse the spectra of pure feces, ingesta and uncontaminated carcass skin and for multivariate analysis. The spectral data set ($N$ = 140 and 48 uncontaminated hard and soft scald, respectively, breast skin; $N$ = 42 duodenum; $N$ = 37 ceca; $N$ = 25 colon and $N$ = 21 ingesta) was transformed with multiplicative scatter correction (MSC).[10] Spectra were mean centered and reduced by principal component analysis (PCA).[11]

## Results and discussion

Immersion of poultry carcasses in hot water (scalding) aids in the removal of feathers. Scalding temperature is one of the factors that affect the appearance of carcass skin. Hard scald (57°C) removes the cuticle resulting in a whiter carcass, whereas soft scald (52°C) keeps the cuticle intact resulting in a yellow carcass. Figure 1 shows the average log (1/$R$) spectra of uncontaminated hard and soft breast skin. Skin from soft scalding had higher absorbance than hard scald immersion due to the yellow pigmentation of the cuticle. The visible region (400–700 nm) represents the colours of the skin as well as the muscle pigments of myoglobin. The colour of meat is largely determined by the relative amount of three forms of myoglobin, i.e. deoxymyoglobin, oxymyoglobin, and metmyoglobin[12] at the meat surface. Liu and Chen[13] reported at least seven absorption bands at 430, 440, 455, 545, 560, 575 and 585 nm associated with the changes in the oxidation and denaturation of myoglobin due to cooking and cold storage. Soft scald breast skin had absorption bands at 432 and 556 nm attributed to oxymyoglobin and the shoulder at 485 nm to metmyoglobin (Figure 1). Hard scalded skin also had bands attributed to oxymyoglobin at 544 and 570 nm.

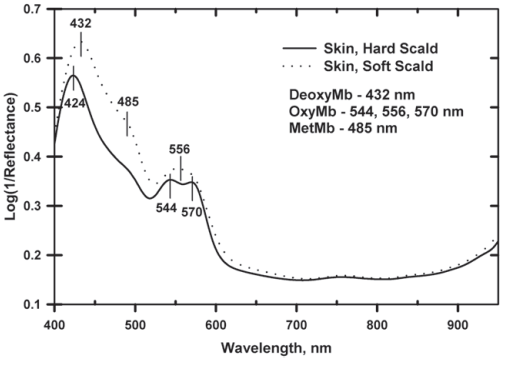


**Figure 1. Average absorbance (log 1/*R*) spectra of hard and soft scald uncontaminated breast skin.**

The appearance of two bands compared with one oxymyoglobin band in soft scalded

skin was possibly due to a change in the secondary structure of the heme groups in myoglobin due to higher immersion temperature. The band at 424 nm can be assigned to the Soret absorbance band for oxymyoglobin.[14]

The feces spectra represent a mixture of plant pigments with broad absorbance, which increased with decreasing wavelength (Figure 2). Fecal and ingesta obtained from the four sampling sites varied greatly in colour. In general, cecal and colon feces were brown to dark brown in colour compared to duodenum feces and ingesta that was yellow–orange to light brown. Feces and ingesta spectra decreased in absorbance at 458 nm as fecal material became browner. In contrast, feces and ingesta had a broad absorbance characteristic (525–700 nm), which increased as the sample became darker (yellow–orange to dark brown).

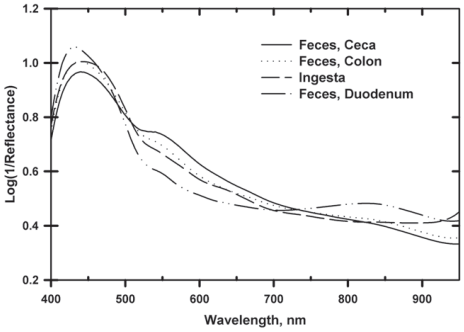


**Figure 2. Average absorbance (log 1/*R*) spectra of pure feces and ingesta.**

The ceca samples were the brownest of the feces and had the greatest absorbance (525–700 nm), whereas the duodenum samples were the lightest in colour of the feces and had the lowest absorbance. In general, the feces and ingesta spectra had greater absorbance than uncontaminated breast skin samples in this region (Figure 1).

Prior to PCA, the spectral dataset was transformed with MSC to remove interferences of light scatter from the skin and differences in pathlength due to pure feces and ingesta sample thickness variations. The first four components accounted for 98% of the total spectral variation, with components 1, 2 and 4 expressing 92% of the variation. Scores from the first PC separated the uncontaminated breast skin from contaminates (Figure 3). Uncontaminated breast skin had negative scores, whereas feces and ingesta had positive scores. Scores from PC2 and 4 partially separated fecal type with some overlap, whereas ingesta was clearly separated from feces. Fecal type was distributed along the axes of the second PC, indicating its wide variation. In addition, there was less spectral variation in feces from the duodenum compared to ceca and colon feces. In an overall evaluation, the first PC is clearly related to the uncontaminated skin, while the second and fourth PCs describe the variations of fecal type and ingesta.

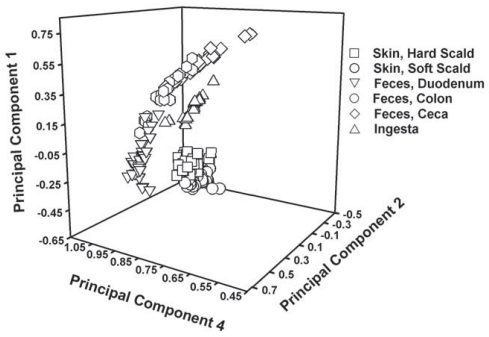


**Figure 3. PCA score plot of components 1, 2 and 4 for uncontaminated hard and soft scald breast skin and duodenum, ceca, colon feces and ingesta.**

These results demonstrate the ability to discriminate between uncontaminated skin and feces due to the variations in the visible/NIR spectra, which arise from the intrinsic differences in colour and chemical constituents of the samples.

Loadings are the regression coefficients of each variable (wavelength) for each PC. Loadings often resemble the spectra of samples and the spectra of constituents and thus offer scope for interpretation of maximum weighting matching known absorbance bands. The loadings of the

PCs, used to discriminate between uncontaminated breast skin and contaminates are shown in Figure 4. The loading plot indicates how the variance is accounted for in a PC across the wavelength scale. Numerically higher (+/-) weights indicate a relative high contribution of the wavelength area to that PC.

The shape of the plot for the first PC showed the broad absorbance (628 nm) characteristic and the inverse relationship of feces and ingesta colour compared to the colour of uncontaminated breast skin. Uncontaminated breast skin had lower absorbance (Figure 1) than contaminates (Figure 2) in this wavelength region. Weights 2 and 4 had large intensities at 565 and 434 nm, respectively, related to the myoglobin and/or hemoglobin of the breast skin. Weight 4 also had significant intensity at 517 nm possibly related to colour differences.

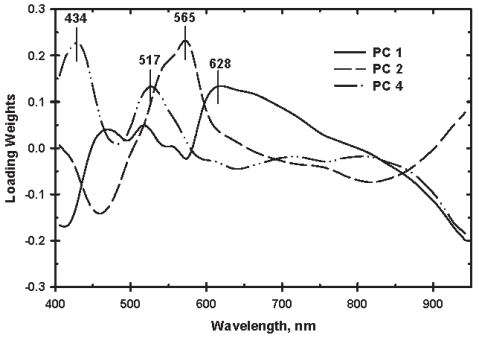


**Figure 4. Loading weights of principal components 1, 2 and 4, for uncontaminated poultry breast skin and contaminates.**

## Conclusions

Visible/NIR spectroscopy can be used to discriminate between uncontaminated poultry breast skin and feces and ingesta from different portions of the digestive tract. Visual assessment of the loadings suggests that discrimination was dependent on the spectral variation related to contaminate colour and myoglobin and/or hemoglobin content of the uncontaminated breast skin. Key wavelengths were identified by intensity of loadings at 628 nm for PC1, 565 nm for PC2 and 434 and 517 nm for PC4. These key wavelengths were selected and applied to hyperspectral images of uncontaminated and fecal contaminated poultry carcasses as described by Park *et al*.[9]

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