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Effectiveness of Hyperspectral Imaging System for Detecting Cecal Contaminated Broiler Carcasses

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Abstract: Broiler processing may result in fecal contamination of the surfaces of carcasses. Fecal contaminants on broiler carcasses are prohibited due to the potential presence of bacterial pathogens. The objective of this study was to determine the effectiveness of the hyperspectral imaging system to detect cecal contamination of known mass. On each of three replicate sample days, twenty-four eviscerated, pre-chilled broiler carcasses were collected from a commercial processing plant. Broiler carcasses were cut longitudinally into contra-lateral halves using a sanitized saw. Cecal contents from the same flock were also collected and used to contaminate carcass. Contents of multiple cecal were combined, homogenized and used to contaminate carcass. Carcass halves were imaged uncontaminated and cecal contents (10, 50, or 100 mg) were applied to the carcass half, and then re-imaged. Cecal detection results varied due to contaminate detection threshold. The imaging system correctly identified 100% cecal mass applied at a threshold of 1.00 and 1.05 but also incorrectly identified 252 and 65 carcass features, respectively that were not contaminates (false positives). False negative were only associated with the 10mg mass and a detection threshold of 1.10. The percentage of true positive cecal pixels (ie. ground truth) detected also varied due to the detection threshold. Averaged across cecal mass, the percentage of the cecal ground truth detected was 74, 55 and 35% for the 1.00, 1.05 and 1.10 threshold, respectively. The percentage of contaminated pixels not detected were a spectral mixture of cecal and uncontaminated skin. Detection of mixed pixels in small contaminants (ie. 10mg and less) or an aggregate of several single-pixels is essential for contaminant identification. Detection of mixed pixels in large contaminants is not significant to overall contaminant identification.

Key words: Hyperspectral, imaging, poultry, feces, food safety

Introduction

Intestinal contents on broiler carcasses are a food safety hazard because of a correlation between feces and pathogenic bacteria. During the course of slaughter and processing, there are opportunities for the alimentary tract, from crop to colon, to leak or rupture, spilling contents onto the skin or muscle of broiler carcasses. Once on the surface of a carcass, such contamination has the potential to persist on the carcass through much of processing (Byrd *et al*., 2002). As a result, the Food Safety Inspection Service (FSIS) established a zero-tolerance policy regarding visible fecal material on poultry carcasses (USDA, 1996). The regulation requires that no carcass can have visible fecal contamination prior to entering the immersion chiller tank in order to prevent cross-contamination among carcasses. The current method of inspecting for fecal contamination is through human visual observation with the criteria of color, consistency, and composition used for identification.

The USDA's Agricultural Research Service has developed hyperspectral and multispectral imaging techniques to detect fecal contaminants on poultry carcasses. (Lawrence *et al*., 2003a; Park *et al*., 2002a; Windham *et al*., 2003a). Research has been conducted on calibration of hyperspectral images (Lawrence *et al*., 2003b), characterization of fecal visible and near-infrared spectra (Windham *et al*., 2003b), fecal algorithm development (Liu *et al*., 2003; Park *et al*., 2004; Windham *et al*., 2003c), image processing (Lawrence *et al*., 2003a) and on-line multispectral application (Park *et al*., 2002b). Fecal detection by hyperspectral and multispectral imaging techniques reported in the above studies has been through contamination of carcasses with ingesta, duodenum, cecal and colon material varying in size and location on the carcass. The effectiveness of the hyperspectral imaging system to detect intestinal contents of known mass has not been reported.

Samples from this study were use by Berrang *et al*. (2004) to determine the effect of cecal contents (2 to 100 mg) applied to broiler carcasses on bacterial counts. Numbers of *Campylobacter, Escherichia coli*, coliforms, and total aerobic bacteria were obtained on carcass halves with and without the addition of cecal contents. Carcass halves with 5 mg or more of cecal contamination had significantly higher numbers of *Campylobacter* than those without ($P < 0.01$). Carcass

halves contaminated with only 5 mg of cecal contents had an average of log 3.3 CFU *Campylobacter* per ml of rinse while corresponding uncontaminated carcass halves had log2.6 CFU *Campylobacter* per ml rinse. These data indicate that even small (5 mg) amounts of cecal contents can cause a significant increase in the numbers of *Campylobacter* on eviscerated broiler carcasses. The objective of this study is to determine the effectiveness of the hyperspectral imaging system to detect cecal contamination of known mass.

Materials and Methods

Samples: Broiler carcasses were collected directly from the shackle line after the inside outside washer in a commercial broiler processing plant as described by Berrang *et al.* (2004). For hyperspectral imaging, twenty four carcasses were collected on 3 replicate sample trips (n = 72). Concurrent with carcass collection, intestinal tracts were collected by manual removal from the processing line immediately after evisceration. Intestinal tracts were gathered and pooled together in a clean plastic bag.

Carcasses were cut in half with a sanitized bone saw along the dorsal/ventral midline resulting in mirror image halves. Each half was placed into a separate clean plastic bag which was numbered to allow identification of halves from the same carcass. One half of each carcass was randomly selected to be contaminated with cecal contents for imaging and microbiological studies (Berrang *et al.*, 2004) while the other half of the same carcass remained uncontaminated as a negative control.

Hyperspectral Imaging System: The hyperspectral imaging system described Lawrence *et al.* (2003a) was used to image uncontaminated and cecal contaminated carcass halves. Briefly, the imaging system consists of an imaging spectrograph with 25-mm slit width - Grating Type I (Im Specter V9, Spectral Vision, Ltd.); a high resolution CCD camera (SensiCam, Cooke Corp.); 1.4/23 mm compact C-mount lens, (Xenoplan, Schneider) and associated optical hardware; motor for lens motion control (Newport); frame-grabber (12-bit PCI interface board, Cooke Corp.); and computer (Pentium III, 500 MHZ). The spectrograph has a nominal spectral resolution of 2.5 nm. It is connected to a 2/3" silicon based CCD sensor with a 1280 x 1024 pixel resolution. For normal illumination of poultry carcasses, two 150-watt tungsten-halogen DC stabilized fiber-optic illuminators (Fiber-Lite A240, Dolan-Jenner, Inc.) were used. Lighting requirements (source and configuration) were adjusted for quality image acquisition.

Procedures: Cecal contents and application: In all experiments, cecal were isolated from the intestinal tract and their contents manually pressed out into a clean plastic dish. Cecal contents were pooled and homogenized by vigorous stirring with a sanitized spatula then packed into sterile 5 ml syringes. The syringe was weighed on an analytical scale, cecal contents were applied to one half of the carcass and the syringe was weighed again. In this way, the mass of cecal contents applied could be calculated. Target mass was 10 mg, 50 mg and 100 mg. Cecal contents were applied to unbagged carcass halves hanging by the legs in a shackle. After application of the cecal mass and image acquisition, each carcass half was replaced in the same plastic bag. In each replication, 8 carcasses were treated by application of the same approximate mass of cecal contents.

Hyperspectral Imaging: At the beginning of each day's imaging, HyperVisual software (provision Technologies, Stennis Space Center, MS) was used to collect system noise (ie. dark current), 99% reflectance panel, and gradient panel measurements for percent reflectance calibration and validation. Once calibration measurements were completed, a carcass or carcass half was hung on a standard evisceration shackle, which was welded to a stainless steel support rod, and imaged immediately. Black cloth was hung behind the bird to provide contrast between the bird and background. HyperVisual software was used to control the camera, which was set at 4 by 2 binning resulting in 320 horizontal spatial pixels and 512 vertical spectral pixels measured per line-scan image. The exposure time was 50 ms. and it took about 40 s. to collect a 400 line-scan image (vertical spatial) needed to image an entire carcass half. After an uncontaminated ("clean") carcass was imaged, a single cecal target mass was applied as one contaminant spot to the thigh of the carcass half.

The clean carcasses and the application of cecal contents were video taped so that the exact location of the contaminant was documented. While video taping the clean carcass, a poultry scientist verbally documented any unusual features on a particular carcass. Some of the items noted on the "clean" carcasses were the locations of feathers, blood clots / hemorrhages, bruises, cuticle, scabs, and numerous other abnormalities.

Image processing: Image hypercubes of "clean" and contaminated carcass halves were calibrated to percent reflectance values as described earlier (Lawrence *et al.*, 2003b). The reflectance data were also spectrally smoothed by boxcar averaging over a 20-nm bandwidth with a custom program written in IDL (Interactive Data Language, Research Systems Inc.), which was compiled and run from within ENVI (Research Systems Inc.). ENVI was the software used for image processing and analysis. The following steps were performed on

Table 1: Summary of detection accuracies averaged over cecal mass

Threshold	Detection accuracy (%)	Total false negatives (n)	Total false positives (n)	Carcasses with FP[a]
1.00	100	0	252	83.7
1.05	100	0	65	22.2
1.10	94	4	8	11.1

[a] Percent carcasses with false positives averaged over cecal mass

Table 2: Mean percent (n = 24) ground truth detection accuracy and 95% confidence intervals at different detection thresholds from broiler carcass contaminated with cecal contents

Cecal mass (mg)	Pixel Contaminant ground truth (n)	1.0 threshold accuracy	1.05 threshold accuracy	1.1 threshold accuracy
9.9±0.7	59.5±12.7	73.3±7.0	51.4±4.9	32.6±12.2
49.0±1.7	169.9±24.5	75.2±4.9	55.4±5.9	36.4±7.7
99.6±2.7	272.0±39.7	73.7±5.4	59.1±5.2	36.3± 6.7

each smoothed image. The background was removed from the carcass image by applying a background threshold mask with a value of 6% reflectance. Next, a ratio image was created by dividing a 565-nm image by a 517-nm image. The background mask was then applied to the ratio image. The ratio of reflectance values at 565 and 517 nm has been determined earlier to be well suited for the detection of fecal contaminants (Park *et al.*, 2002a). Typically, feces and ingesta reflectance spectral data increase with frequency from 420 nm to 708 nm whereas, spectra of skin, meat, and bones decrease from 500 to 560 nm (Lawrence *et al.*, 2003c). Therefore, dividing an image at 565 nm by an image at 517 nm would result in cecal contaminants with values greater than one while non-contaminated skin would have values less than one. To test the effectiveness of the HIS for detecting cecal contaminants varying in mass, thresholds of 1.0, 1.05, and 1.10 were applied to the masked-ratio image to delineate the cecal contaminants from the remainder of the image. Finally a 3 x 3 median filter was applied to remove speckled noise (Mather, 1999).

In addition, on each carcass image, true positive pixels of cecal contaminants were selected using the rectangle, polygon, and drawing point modes of ENVI software as regions of interest (ROI). The video tape was used as an aid for selecting ROIs by referencing the video to the image for each cecal mass. The ROI were used to determine the number of true positive pixels in a contaminant mass (ie. ground truth) and compare calculate detection accuracy results from applied contaminant thresholds.

Results and Discussion

Table 1 shows the detection accuracy averaged over cecal mass at each contaminant threshold evaluated. The hyperspectral imaging system correctly detected 100% of the cecal spots applied to the carcass halves at a threshold of 1.00 and 1.05. However, at a threshold of 1.00, 256 false-positives contaminants (detected as contaminant but no contaminant present) were incorrectly identified on 84% of the carcass halves. Lawrence *et al.* (2003c) reported 196 false positive on 60% of carcasses when detecting duodenum, cecal, colon and ingesta contaminants at a threshold of 1.00. Fig. 1b summarizes the sources of false positives contaminants at a 1.00 threshold. Yellow skin was the largest source of false positives in this study where as Lawrence *et al.* (2004) reported scabs or old wound as the prevalent false positives. In contract only 65 false positives were incorrectly identified on 22% of the carcass halves at a threshold of 1.05. The false positives were scabs, skin, boundary and shadow features (Fig. 1b). One carcass half had 72% of the scab false positives and the remaining scab and other features were in the single- to five-pixel errors distributed among 15 carcasses.

False negatives were only associated with the 10 mg cecal mass applied to 4 carcass halves and a contaminant threshold of 1.10 (Table 1). The 10 mg cecal mass is small in size and the low concentration made detection difficult at the 1.10 threshold. In addition, the 10 mg contaminant mass had a high proportion of spectrally mixed pixels of skin and cecal, which also made detection difficult. Single pixel false positive scab and boundary features were distributed among 11% of the carcass halves. Given the large number of false positives with the 1.00 threshold and the false negatives with the 1.10 threshold, the 1.05 contaminant threshold is optimal for this study.

For each carcass image, ROI's were selected (Windham *et al.*, 2003c) of cecal mass applied to the carcass and used to determine the number of true positive pixels of the cecal mass (ie. ground truth). The next to last step in processing images is an image of the masked ratio with a number of contaminant pixels detected based on the threshold applied. As such, the ground truth pixels can be used to determine the percentage of the cecal mass detected. The pixel contaminant ground truth and detection accuracies are

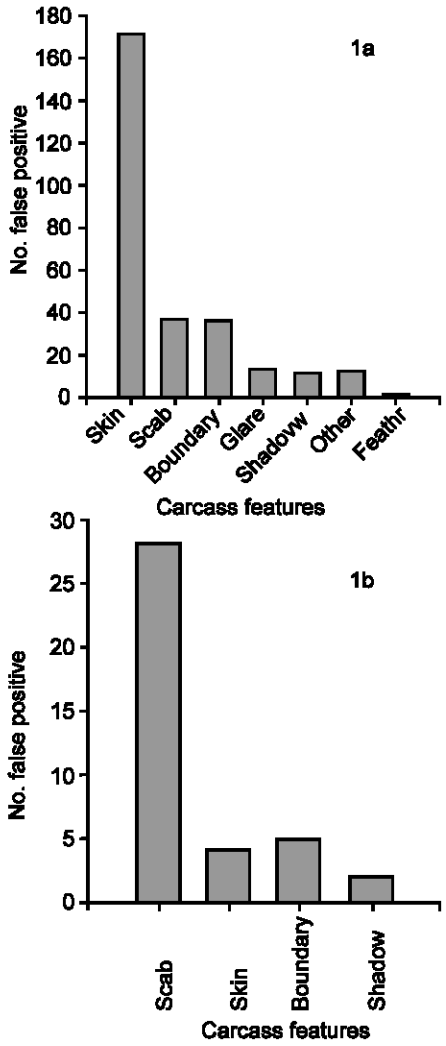

Fig. 1: Bar graph of false positives identified by hyperpsectral imaging system; 1a 1.00 contaminant threshold, 1b 1.05 contaminant threshold.

shown in Table 2. The number of ground truth pixels averaged 59, 170, and 272 for cecal mass 10, 50, and 100 mg, respectively. The variation in ground truth pixel number was greater than cecal mass due the difficulty of evenly applying the contaminant to the carcass. At a given threshold, there was little difference in detection accuracy due to cecal mass. The high 95% confidence interval for 9.9 mg cecal mass and 1.10 threshold was due to the 4 false negatives. Averaged across cecal mass, the detection accuracy was 74, 55, and 35% for the 1.00, 1.05, and 1.10 contaminant threshold, respectively.

Using the same samples, Berrang *et al.* (2004) reported that applying cecal contents in amounts of about 100 or 50 mg resulted in significant increases in all bacterial populations measured with the biggest increase being noted with *Campylobacter*. Application of approximately 10 mg cecal contents did not result in significantly higher numbers of total aerobic bacteria, but did cause a significant increase in the numbers of *E. coli*, coliforms and *Campylobacter*. In replicate 2, the microbiological method was unable to distinguish between carcass halves contaminated with a 10 mg mass and the corresponding uncontaminated halves ($P<0.05$). Coliform counts averaged 3.85 per ml of carcass rinse (log10) for contaminated carcasses and 3.83 for uncontaminated carcasses, while *E. coli* counts were 3.83 vs. 3.41, respectively. The hyperspectral imaging system detected 100% of the 10 mg contaminants and appeared to be more effective than microbiological method for detecting small amounts of contamination.

Fig. 2 shows an image of a 90.0 mg cecal contaminant and the effect of the applied contaminant threshold on true positive cecal pixels not detected (black) and detected (gray or yellow). The combination of pixels not detected and detected equals the ground truth or the total number of pixels covered by the 90.0 mg contaminant. The 1.10, 1.05, and 1.00 threshold (Fig. 2b,c, and d, respectively) correctly detected 46, 59, and 78%, respectively of the ground truth. Cecal not detected with a 1.10 and 1.05 threshold were pixels within the contaminant that did not completely cover the skin (Fig. 2a) and pixels on the boundary of the contaminant. This example represents the effect of the contaminant threshold on pixels not detected and detected in this study.

The hyperspectral imaging system and algorithms developed (Lawrence *et al.*, 2003a, 2003b; Park *et al.* 2002a; Windham *et al.* 2003b) uses images at 565 and 517 nm to detect fecal contaminants. The skin spectra (Fig. 3) tended to have a higher reflectance than contaminants and have peaks around 512 and 560 nm that have been associated with the oxidative state of the myoglobin in the skin (Windham *et al.*, 2003b). Typically, fecal material or in this case cecal detected (Fig. 3) have spectra that increase in reflectance from 420 nm to 780 nm whereas, most other spectra (skin, meat, bones, blood, etc.) decreased from 500 to 560 nm. Therefore, dividing an image at 565 nm by an image at 517 nm would result in contaminants with values greater than one while non-contaminants would have values less than one. Spectra from pixels on the boundary of the cecal contaminant not detected (Fig. 3) are a mixture of the cecal and skin as indicated by the reflectance peaks for myoglobin in the skin. Mixed pixels are problematic to detect with 565/517-nm ratio regardless of the contaminant type (ie. ingesta, duodenum, colon, cecal) because the wavelength values are too close to each. The detection of mixed pixels is not critical to detection with cecal contamination greater than 10 mg. However, smaller amounts of contamination would be difficult to

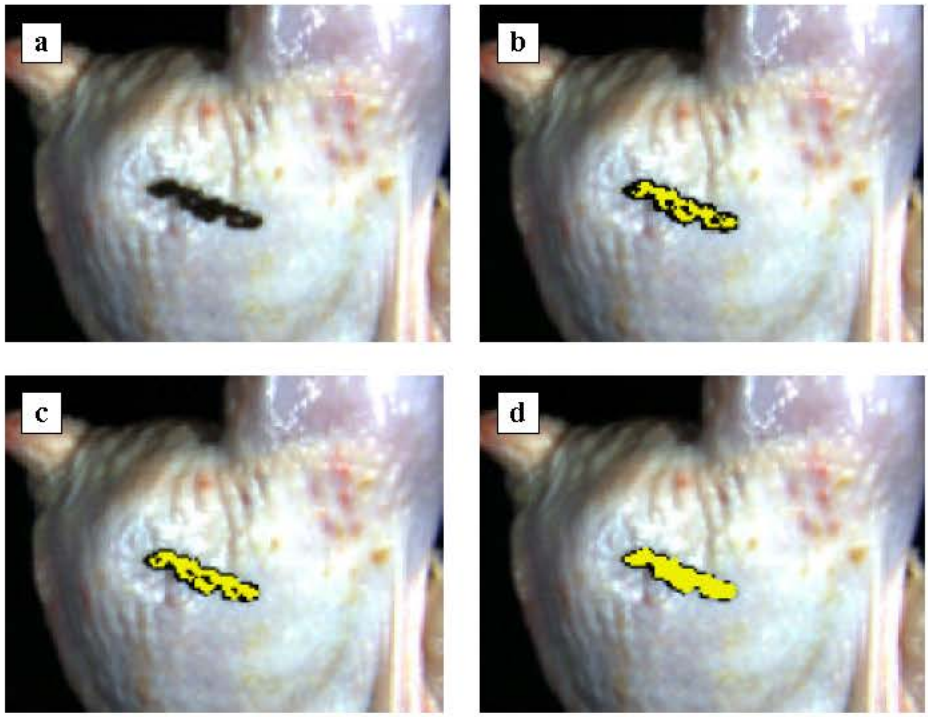

Fig. 2: Color composite image of a 90 mg cecal mass contaminant (a) and pixels not detected (black) and pixels detected (gray or yellow) with a 1.10 threshold (b), a 1.05 threshold (c), and a 1.10 threshold (d).

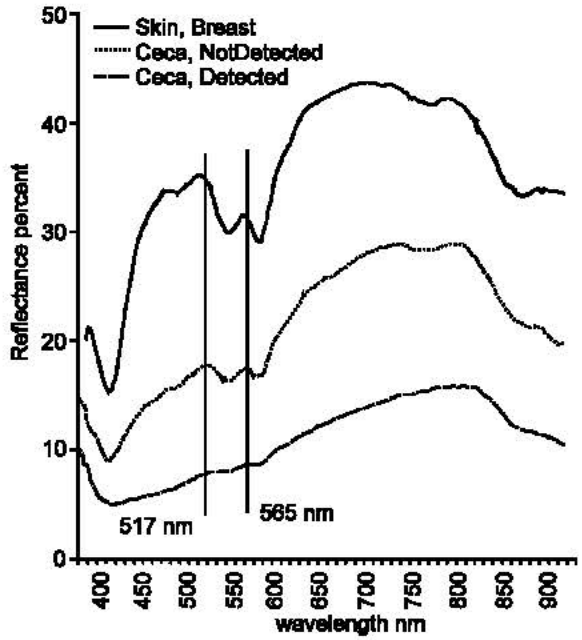

Fig. 3: Typical mean spectra of poultry breast skin and cecal contaminant. Mean spectra spatially averaged over cecal detected and cecal edge pixels not detected (Fig. 2c).

detect because a greater proportion of the ground truth pixels are mixed pixels.

Conclusions: A hyperspectral imaging system was used to detect cecal contaminants of known mass applied to broiler carcass halves. The imaging system identified 100% of the 10, 50, and 100 mg cecal contaminants applied with a contaminant threshold of 1.05. The hyperspectral imaging system appeared to be more effective the traditional microbiological method for detecting 10 mg contaminants. A threshold of 1.00 correctly identified the contaminants but also incorrectly detected a large number of carcass features as false positives. A threshold of 1.10 did not identify some the 10 mg contaminants. The percentage of true positive cecal pixels detected varied due to the contaminant threshold. Contaminated pixels not detected were a spectral mixture of cecal contents and uncontaminated skin. Detection of mixed pixels in large contaminant is not significant to overall identification. However, detection of mixed pixels in a small contaminant (ie. less then 10 mg) or an aggregate of several single-pixels is essential for identification. Further research is needed on the detection of small contaminants and the analysis of mixed pixels on a pixel-by-pixel level.

References

Berrang, M.E., D.P. Smith, W.R. Windham and P.W. Feldner, 2004. Effect of intestinal content contamination on broiler carcass *Campylobacter* Counts. J. Food Protection., 67: 235-238.

Byrd, J.A., B.M. Hargis, D.E. Corrier, R.L. Brewer, D.J. Caldwell, R.H. Bailey, J.L. McReynolds, K.L. Herron and L.H. Stanker, 2002. Fluorescent marker for the detection of crop and upper gastrointestinal leakage in poultry processing plants. Poult. Sci., 81: 70-74.

Lawrence, K.C., W.R. Windham, B. Park and R.J. Buhr, 2003a. Hyperspectral imaging system for identification of faecal and ingesta contamination on poultry carcasses. J. Near Infrared Spectrosc., 11: 261-281.

Lawrence, K.C., B. Park, W.R. Windham and C. Mayo, 2003b. Calibration of a pushbroom hyperspectral imaging system for agricultural inspection. Trans. ASAE., 46: 513-521.

Lawrence, K.C., W.R. Windham, D.P. Smith and B. Park, 2003c. Effect of broiler carcass washing on fecal contaminant imaging. ASAE Technical Paper No. 033122. ASAE, St. Joseph, MI.

Liu, Y., W.R. Windham, K.C. Lawrence and B. Park, 2003. Simple algorithms for the classification of visible/NIR and hyperspectral imaging spectra of chicken skins, feces, and fecal contaminated skins. J. Appl. Spectroscopy., 57: 1609-1612.

Mather, P.M., 1999. Computer processing of remotely-sensed images. John Wilely & Sons, Chichester, England.

Park, B., K.C. Lawrence, W.R. Windham and R.J. Buhr, 2002a. Hyperspectral imaging for detecting fecal and ingesta contamination on poultry carcasses. Trans ASAE., 45: 2017-2026.

Park, B., K.C. Lawrence, W.R. Windham and D.P Smith, 2002b. Multispectral imaging system for fecal and ingesta detection on poultry carcasses. ASAE Paper No. 023138, Am. Soc. Agri. Eng., St. Joseph, MI.

Park, B., W.R. Windham, K.C. Lawrence and D.P. Smith, 2004. Hyper spectral image classification for fecal and ingest identification by spectral angle mapped. ASAE Technical Paper No. 043032. ASAE, St. Joseph, MI.

Windham, W.R., K.C. Lawrence, B. Park, L.A. Martinez, M.A. Lanoue, D.A. Smith, J. Heitschmidt and G.H. Poole, 2003a. Method and system for contaminant detection during food processing. U.S. Patent No. 6587575.

Windham, W.R., K.C. Lawrence, B. Park and R.J. Buhr, 2003b. Visible/NIR spectroscopy for characterizing fecal contamination of chicken carcasses. Trans ASAE., 46: 747-751.

Windham, W.R., D.P. Smith, B. Park and K.C. Lawrence, 2003c. Algorithm development with visible/near infrared spectra for detection of poultry feces and ingesta. Trans. ASAE., 46: 1733-1738.

USDA, 1996. Pathogen reduction, hazard analysis and critical control point (HACCP) systems. Final Rule. Fed. Reg., 61: 28805-38855.

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