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Using Hyperspectral Fluorescence Spectra of Deli Commodities to Select Wavelengths for Surveying Deli Food Contact Surfaces

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Abstract

Purpose: The inability to adequately judge the efficacy of cleaning and sanitation procedures in deli departments is a recognized food safety concern. In a prior study, our research group demonstrated that visual inspection of cleaned produce processing surfaces could be enhanced through the use of a portable fluorescence imaging device that detected residual produce residues. **Methods:** To explore the feasibility of using fluorescence imaging to similarly detect residual deli residues, spectra of American, Cheddar, Provolone, and Swiss cheeses and of processed chicken, ham, roast beef, and turkey were acquired using a laboratory hyperspectral imaging system. Circular punches of these commodities were placed onto stainless steel and high density polyethylene coupons for imaging. The coupon materials were selected to represent common surfaces found in deli departments. **Results:** Analysis of hyperspectral fluorescence images showed that cheeses exhibited peaks in the blue-green region and at around 675 nm. Meats exhibited peaks in the blue-green region with one of four ham and one of four chicken brands exhibiting peaks at around 675 nm, presumably due to use of plant-derived additives. When commodities were intermittently imaged over two weeks, locations of spectral peaks were preserved while intensity of peaks at shorter wavelengths increased with time. **Conclusion:** These results demonstrate that fluorescence imaging techniques have the potential to enhance surface hygiene inspection in deli departments and, given the immediate availability of imaging results, to help optimize routine cleaning procedures.

Keywords: Cleaning, Deli, Fluorescence imaging, Food safety, Sanitation

Introduction

Food safety is a top priority in the food industry. Ready-to-eat (RTE) foods including fresh deli commodities pose a high risk for causing foodborne illness. For example, deli meats are recognized as the leading cause of listeriosis in the United States, causing an estimated 1,600 illnesses per year and accounting for roughly 64% of all listeriosis cases. Furthermore, when compared to prepackaged deli meats, freshly sliced meats from retail facilities accounted for approximately 83% of deli meat listeriosis cases, causing approximately 167 deaths per year (USDA FSIS, 2010). An interagency report on risk assessment for *Listeria monocytogenes* in retail delicatessens concluded that "sanitation practices were a key driver in reducing the predicted risk of listeriosis" (FDA, 2013).

Current sanitation verification in deli departments is primarily based on human visual inspection. This technique allows for a broad inspection of food-contact surfaces in real time, but cannot fully verify the efficacy of cleaning and sanitation efforts. Alternative methods for verification include ATP testing and culturing for pathogen detection (Cunningham et al., 2010). While these alternate methods are more sensitive than visual inspection, they are costly, time consuming, and can address only limited subsets of actual surface areas in a deli. Recently, it was demonstrated that fluorescence detection of produce residues

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using a portable imaging device can be used to increase the efficacy of visual inspection after cleaning and sanitation in produce processing plants (Lefcourt et al., 2013; Wiederoder et al., 2013). The device allows most processing surfaces to be inspected, and the immediacy of results allows problem areas to be identified and reprocessed. In use, identification of problematic areas allowed cleaning protocols to be modified to be more effective with no overall increase in time expenditure. A critical step in the development of procedures for surveying produce processing plants was identification of a limited number of wavelengths to be used for routine scanning (Wiederoder et al., 2012). Using visible-wavelength data acquired using a laboratory hyperspectral imaging system, this study established that produce residues could be detected using fluorescence imaging and identified three wavelengths for testing in processing plants. A hyperspectral imaging system generally allows creation of 3-dimensional data sets where two dimensions encompass the coordinates of individual image pixels and the third dimension is intensity by wavelength, i.e., the spectra for each pixel location (Kim et al., 2011). Fluorescence imaging was used for detection due to sensitivity for detecting low concentrations of biological compounds of interest (Kim et al., 2012; Wiederoder et al., 2012). Similarly, the goal of this study is to establish that deli commodity residues can be detected using fluorescence imaging and to identify wavebands for further testing. Secondary goals are to examine possible changes in fluorescence spectra of deli residues as the residues age, and whether it might be possible to use imaging of fluorescence responses to classify residues as being meat or cheese.

Materials and Methods

A laboratory hyperspectral system was used to acquire fluorescence images of deli commodities obtained from deli departments in local grocery stores. Spectra of acquired images were visually analyzed with the goal of identifying wavelength bands suitable for use by a fluorescence imaging device to enhance real time visual inspection of deli devices and surfaces after cleaning and sanitation.

Hyperspectral imaging system

The laboratory line-scan hyperspectral imaging system has been described (Kim et al., 2011; Wiederoder et al., 2012). The system includes an electron-multiplying charge-coupled device (EMCCD) camera (MegaLuca R; ANDOR Technology, South Windsor, CT), an imaging spectrograph (VNIR Concentric Imaging Spectrograph; Headwall Photonics, Fitchburg, MA), a C-mount lens (F 1.9, 35-mm compact lens; Schneider Optics, Hauppauge, NY), and a 405 nm LED (LZ4-40UA10, LED Engin., Inc., Santa Clara, CA) light source. Software developed in-house using Microsoft Visual Basic Version 6.0 (Microsoft, Seattle, WA) was used to acquire and to analyze images. Vertical pixels (spectral dimension) were binned by four to produce 85 waveband intervals from 460 to 800 nm at approximately 4 nm intervals. Horizontal pixels were binned by two to yield a spatial resolution of approximately 0.5 mm per pixel. To match the spatial resolution of line-scan images, the translation table was increment in 0.5 mm steps. Images were corrected for dark current.

Sample preparation

Slices of American, Cheddar, Provolone, and Swiss cheese and processed chicken, ham, roast beef, and turkey were acquired from deli departments of local grocery stores. Commodities for testing were selected based-on popularity and, thus, the likelihood of generating residues. All commodities were sliced to similar thickness (~5 mm) and imaged on the same day as purchase. The first and last slices of each deli commodity were discarded. A cork borer was used to create 2.5 mm radius punches. Thirty-two replicate punches of each commodity were placed both on a stainless steel (SS) coupon and on a high-density polyethylene (HDPE) coupon. Coupon materials were selected to represent surfaces commonly encountered in deli departments. The cork borer was wiped free of debris and then rinsed with water between each commodity. The coupons were virgin material, and were washed with soap and water and then rinsed prior to use.

Two additional coupons, one SS and one HDPE, were prepared using all cheese types; eight punches of each cheese type were placed randomly on each coupon. A corresponding set of coupons was created for the processed meats.

In a subsequent trial to examine possibly changes in spectra over time, slices of three additional brands of each type of cheese (American, Cheddar, Provolone, and Swiss) and of processed meats (chicken, ham, roast beef, and turkey) were acquired from at least two different grocery store deli departments. Sampling was handled as

described above. Eight SS coupons (representing the 8 different commodity types) were populated so that each coupon contained three columns (representing the three brands of a selected commodity) and three rows (representing the three replicates of each brand) of punches. Coupons were repeatedly imaged over a period of 14 days, on days 0, 1, 2, 4, 8, 11, and 14. Between measurements, coupons were stored at room temperature is sealed polyethylene containers.

Image analysis

Software was used to generate and automatically place the appropriate number of circular regions of interest (ROI; 81 pixels) for each coupon. Automated placement was based-on knowledge of the approximate location of punches and was fine-tuned by determining the "center of mass" of pixel intensities for each punch. Placement was visually verified and manually adjusted if appropriate. As controls, three circular ROI (29 pixels) for each coupon were manually placed away from punches. Data files were created that included an identifier, the location, and the average intensity of each ROI by spectral waveband.

Using the data files, a spectral graph for each of the 32 ROI was created using Excel (2007; Microsoft, Seattle, WA) and the 32 graphs for a selected commodity were overlaid. Due to uniformity of spectra for each commodity, an average spectral graph for each commodity was created across ROI. For multiple-commodity coupons, spectra were averaged across the eight ROI for each commodity. For the controls, spectra were averaged across the three control ROI for each coupon. For coupons imaged over time, averaged spectral graphs were created for each brand of a commodity at each time point.

Waveband selection

Spectral graphs were visually examined for common wavebands by commodity type with the goal of establishing a minimal set of wavebands that allowed detection of all deli residues. In addition, attempts were made to identify wavebands that allowed discrimination among different types of residues, e.g. cheese and meat.

Results and Discussion

Commodity spectra

Mean spectra with standard errors for cheeses and for meats on SS coupons are shown in Figures 1 and 2, respectively. Mean spectra were created using average intensities for ROI for the 32 replicates of each commodity. For all commodities, fluorescence responses exceeded baseline at all visible wavelengths. For cheeses, broad peaks were seen in the blue-green region with relatively narrower peaks at around 675 nm with the peak for Swiss being less well defined. A small peak at around 630 nm was present for Swiss. For meats, spectral intensities were generally lower than for cheeses; broad peaks were seen in the blue-green region and relatively narrower peaks for ham at round 640 nm and for chicken at around 675 nm.

Background coupon material can impact the magnitude of a fluorescence response (Jun et al., 2010). As has been reported previously for produce residues (Wiederoder et

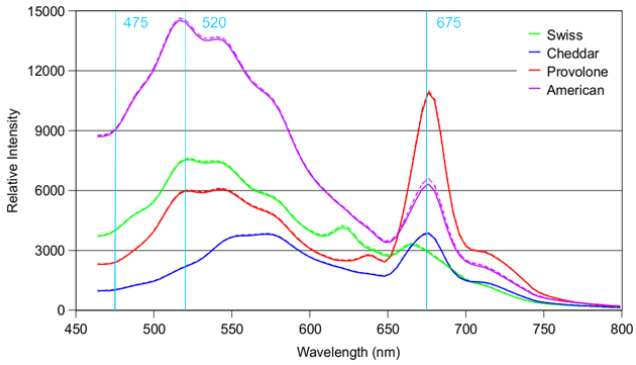

Figure 1. Means and standard errors (dotted lines) of relative fluorescence intensities for the 32 replicate samples of Swiss, Cheddar, Provolone, and American cheeses in response to violet-405 nm excitation. The vertical turquoise lines indicate wavelengths used for imaging in produce plants.

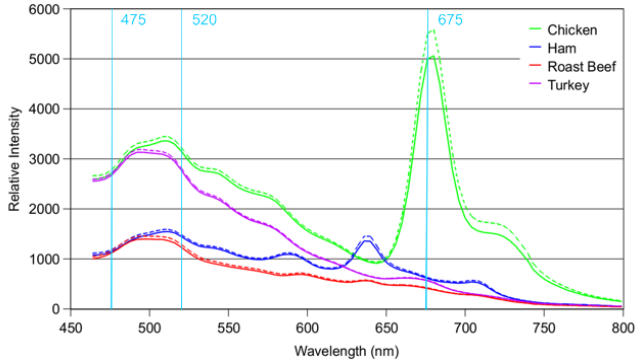

Figure 2. Means and standard errors (dotted lines) of relative fluorescence intensities for the 32 replicate samples of processed chicken, ham, roast beef, and turkey in response to violet-405 nm excitation. Note that intensities measured for processed meats were lower than for cheeses, and that the y-axis range is reduced from that in Figure 1. The vertical turquoise lines indicate wavelengths used for imaging in produce plants.

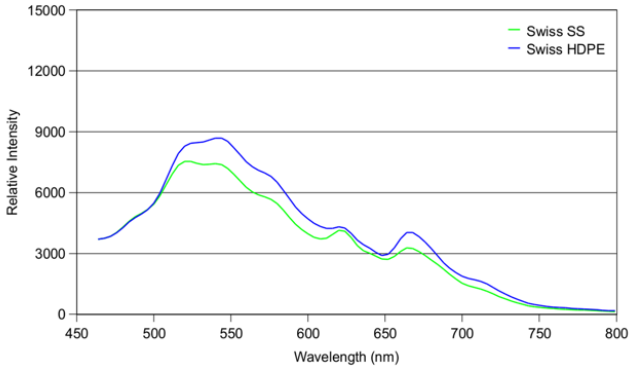

Figure 3. Mean relative fluorescence intensities for Swiss cheese on SS or HDPE coupons. Similar elevated responses for HDPE coupons were seen for all other commodities and are due to the relatively high fluorescence response of HDPE (scattering).

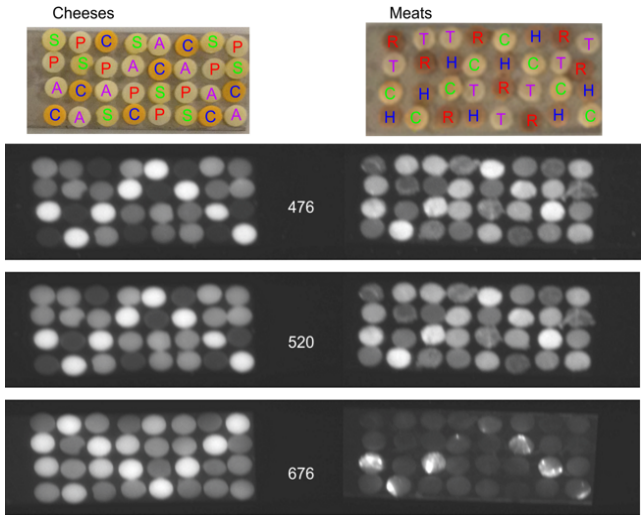

Figure 4. Normalized fluorescence response images of Swiss (S), Cheddar (C), Provolone (P), and American (A) cheeses, and processed chicken (C), ham (H), roast beef (R), and turkey (T) on stainless steel at 476, 520, and 676 nm.

al., 2012), spectra for commodities on HDPE coupons were elevated but were otherwise similar to spectra for the same commodity on SS. It is hypothesized that this difference is due in part to scattering of the response of the highly fluorescent background HDPE as compared to SS. The background coupon effect seen in the example for Swiss cheese was found for all commodities (Figure 3). Because of this consistent relation between spectra when using SS or HDPE coupons, the tests of time and brand effects were conducted using only SS coupons.

Spectral variability within sample punches

Although average intensities within ROI for a given wavelength and commodity were generally consistent, there were cases where areas within an individual punch exhibited relatively higher intensity responses. There are a number of possible reasons for these localized responses. In general, processed meats are less homogeneous than cheeses as meats can contain many different tissue types including muscle, fat, bone, and fascia. These different tissue types can have different fluorescent responses to UV excitation (Kim et al., 2003), and net responses for a local area will depend on the amounts, particle sizes, and distribution of the components within that area. In addition, binders such as soy fiber may also be present (USDA FSIS, 2013). Another possible source of localized responses, for both meats and cheeses, is the use of additives such as herbal spices. The greatest variation in spectra was seen for chicken at around 675 nm (Figure 2). Figure 4 demonstrates that the variability for responses at around 675 nm for individual punches was due to localized areas of intense response within individual punches. Examination of the chicken samples led to the conclusion that the localized effect was most likely due to a binding agent, and the shape of the spectra suggested that the binder contained plant material. Common sources of fluorescence responses at around 675 nm are chlorophyll *a* and related byproducts ubiquitous to plant materials (Chappelle et al., 1991; Murata et al., 1966). In addition, small localized bright spots were occasionally seen for both meats and cheeses, particularly around 675 nm. In two cases the bright spots were found to be what appeared in images to be seeds, presumably used as spices.

Spectral variation over time and across individual commodity brands

Time between preparation of samples and imaging had only a marginal effect on peak wavelengths and no effect on peaks numbers. However, for wavelengths below about 600 nm, the amplitude of spectra increased monotonically with time. As an example, Figure 5 shows fluorescence responses by time for one brand of Provolone cheese. In contrast, Wold et al. (2005) measured fluorescence spectra of Jarlsberg cheese over 48 h. Spectral peak locations were similar to those seen in this study for cheeses; conversely, amplitudes of spectra at wavelengths below 600 nm decreased monotonically with time. Still, the amplitudes of the spectra for the Jarlsberg cheese in the blue-green region remained relatively high. Factors hypothesized to be involved in these changes in amplitudes

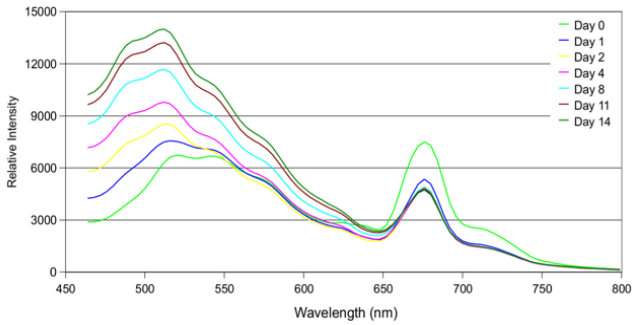

Figure 5. Fluorescence responses of one brand of Provolone cheese on SS by time.

over time include a complex interaction of effects of drying, shrinkage, and oxidation. In any case, results demonstrate that age should not be a significant factor in the ability to detect deli residues using fluorescence imaging techniques.

To examine variation due to brand, day 0 spectra for the three brands of a commodity were compared among themselves and to the corresponding spectra in either Figure 1 or 2. Only for turkey were the four spectra essentially the same. For American and Cheddar cheeses, spectral characteristics were similar with some differences in the absolute amplitudes of peaks across brands. For all the other commodities, there were distinct differences in spectra across brands (Table 1). The characteristic-like chlorophyll *a* peak seen with one brand of ham may be due to an additive used as part of a consumer trend toward "nitrate-free" cured products. To be able to remove added nitrites from the food label and call a product a "no-nitrite-added cured meat", indirect techniques to add nitrites are often used. One technique is to add celery juice or powder, which naturally contain nitrate, and converting some of the nitrate to nitrite using a reducing culture (Sebranek and Bacus, 2007). Since celery contains chlorophyll, the addition of a celery additive would be expected to result in a peak at around 675 nm.

Selection of wavelengths for use in delis

In delicatessens, the substances likely to be on processing surfaces following cleaning and sanitation procedures are residues from cheeses and meats, and sanitizing agents. In order to be able to use imaging techniques to survey the hygiene of processing surfaces subsequent to cleaning and sanitizing procedures, it is critical that the imaging system can detect small quantities of residues and does not detect cleaning and sanitizing agents. In a previous study, tests of common cleaning and sanitizing agents demonstrated that such agents do not fluoresce (Wiederoder et al., 2012). If the ability to detection cleaning or sanitizing solutions becomes an issue, a food-grade fluorescent dye could be added to the solutions.

Current results show that fluorescence responses of tested deli commodities exceed baseline at all visible wavelengths. However, due to similarity of spectra of some commodities, differences in spectra among some brands of the same commodity, and confounding factors

Table 1. Differences in spectra among brands of a commodity and in comparison to the corresponding spectra in either Figure 1 or 2.

Cheeses	
Swiss	Spectra for all three brands were similar to the spectra in Figure 1 except for more pronounced peaks at around 585 nm and 675 nm, and, for two brands, more pronounced peaks at around 630 nm.
Cheddar	Spectra for all three brands were similar to the spectra in Figure 1 with some differences in absolute amplitude of peaks.
Provolone	Spectra were similar to the spectra in Figure 1 except one brand evidenced almost no peak at around 675 nm.
American	Spectra for all three brands were similar to the spectra in Figure 1 with some differences in absolute amplitude of peaks.
Meats	
Chicken	Spectra for all three brands at lower wavelengths were similar to the spectra in Figure 2; however, none replicated the peak seen at around 675 nm.
Ham	Spectra for the three brands and from Figure 2 differed. The peak seen at 640 nm in Figure 2 was not replicated. One brand showed a peak at around 675 nm. There was a local maxima for spectra from all four brands at around 520 nm.
Roast Beef	The three brands showed similar spectra, including peaks at around 590 and 630 nm that were not seen in Figure 2. However, the amplitude of the broad peak in the blue region seen in Figure 2 was much lower for the three additional brands; amplitudes in the blue region did increase with elapsed time after sample preparation.
Turkey	Spectra for all three brands were similar to the spectra in Figure 1.

introduced by the possible use of additives, reliable identification of specific commodities is not feasible when considering use of a survey instrument with a limited number of wavelengths available for scanning. Instead, wavelengths for survey use were selected based on maximizing sensitivity for detection across all test samples without regard to identification of commodities. For inspection following cleaning and sanitation, identification of residues is not required. The ability to detect unidentified residues in real time will allow workers to monitor the efficacy of cleaning procedures, to re-clean as necessary, and to revise cleaning protocols as warranted.

While it is theoretically possible to use the hyperspectral data to develop a classification algorithm for the deli commodities, this was not attempted for two reasons. First, the goal of the study was to identify a limited number of wavelengths for potential use by a survey instrument. Second, any such classification algorithm would have to be assumed to be descriptive rather than predictive given that significant portions of fluorescent responses appeared to be related to the use of additives and vendors can select among a wide variety of additives that could be used during processing.

There are a number of additional factors that need to be considered when selecting potential wavelengths for use in routine monitoring of commercial facilities, including ambient lighting conditions and operating characteristics of the imaging device. In a commercial setting, measurements would likely have to be made with at least minimal ambient lighting and reflected energy from that lighting could mask fluorescence responses. Thus, selection of wavelengths used for routine monitoring should consider the spectra of the ambient illumination, i.e., consideration should be given to wavelength regions where ambient illumination intensity is relatively low. A critical characteristic of any fluorescence imaging device would be the center wavelengths and bandwidths (normally specified as full width at half maximum) of the filters used for imaging. Common bandwidths are 3-4, 10, and 20 nm. Narrower bandwidth filters allow more specificity, while wider filters allow more light to pass. Two other factors to consider are an unexpected fluorescent response from some normal component of the processing environment and the number of wavelengths needed to be able to detect all commodities or contaminants of interest.

One of the reasons our laboratory developed a portable hyperspectral imaging system was to be able to assess issues related to potential use of fluorescence imaging for inspection through tests in actual commercial environments (Lefcourt et al., 2013, Wiederoder et al., 2013). The portable hyperspectral system uses a liquid-crystal tuneable filter with a 20 nm bandwidth and 405 nm LEDs to excite fluorescence responses. For scanning, the system cycles at a selected time interval, normally 1 sec, among any number of pre-selected wavelengths. Cycling can be stopped and started by tapping the display screen. When a problem area is detected, the unit can be mounted on a tripod and a sequence of hyperspectral images acquired at a selected interval, normally 5 nm, from 465 to 700 nm. These efforts were meant to complement ongoing laboratory efforts to develop a cost-effective, commercially-viable, handheld imaging system (Kim et al., 2012, Lee et al. 2013). Practical tests using the hyperspectral device in produce processing plants validated the decision to continuously cycle through three wavelengths, 475, 520, and 675 nm. These three wavelengths are at or near response peaks of contaminants of interest and also corresponded to troughs in plant illumination intensity. The plant used fluorescent lighting, which is ubiquitous in commercial environments. Visual examination of hyperspectral data sets demonstrated that these three wavelengths allowed detection of all contaminants in the produce plant environments that could be detected using the imaging device. Cycling through more than three wavelengths was found to be excessively cumbersome. The device was also used in an attempt to facilitate cleaning of deli slicers (Beck et al., 2015).

Environmental conditions in delis should be similar to conditions in produce plants with regards to ambient lighting and materials used to construct facilities and equipment. Furthermore, the three wavelengths chosen for use in produce plants correspond well with spectral peaks of deli commodities in the current study (Figure 1-2). Thus, the closest corresponding wavelengths for which data were acquired in this study, 476, 520, and 676 nm, were selected for use in visual analysis of images. It should be noted that a 5-10 nm shift in any of the three selected wavelengths should have a minimal impact on detectability of deli residues.

Consideration of acquired spectra and examination of spatial aspects of response images at these three wavelengths (Figure 4) suggests that 520 nm is the best wavelength for detection of deli residues. Responses at 476 nm were similar, but generally a little lower in

intensity. More specifically, the 520 nm region is the only region of overlap in responses of all brands of ham (Table 1). For the 676 nm wavelength region, the intensity of responses for some commodities exceeded those at 520 nm. For example, examination of Figure 4 demonstrates that it would be easier to detect Cheddar cheese at 676 nm. In general, responses of cheeses at 676 nm are comparable to responses at 520 nm while responses of meats are much less intense at 676 nm compared to responses at 520 nm. Food handling protocols for delicatessens emphasize the need to use different slicers for meats and for cheeses; principally because cheeses often contain active cultures that could then contaminate sliced meats (USDEC, 2005). Thus, a goal of this study was to determine whether it might be feasible to classify a residue as being meat or cheese. The relatively low fluorescence responses of most meats at 676 nm can be used to classify responses seen at 520 nm as being from either meat or cheese. However, this method of classification is not 100% accurate, principally due to use of additives. Using this classification method, one of the four brands of ham and one of the four brands of chicken might be incorrectly classified as being cheese due to high intensity responses at around 676 nm. Also, one brand of Provolone cheese and one brand of Swiss cheese might be classified as meat as the intensity of responses at around 676 nm were relatively small.

The commodity that might be the most difficult to detect is roast beef. The two suitable wavelengths for detecting roast beef are 520 and 675 nm. However, three of the four brands of roast beef tested had relatively low intensity responses at these two wavelengths, but all three had a local maxima at around 630-640 nm and images acquired at 636 nm showed better contrast compare to at 520 and 676 nm (Figure 6). One brand of ham and three brands of Swiss cheese also had a local maxima around 630-640 nm. Thus, consideration might be given to using a wavelength around 630-640 nm as the third wavelength for routine monitoring instead of 475 nm.

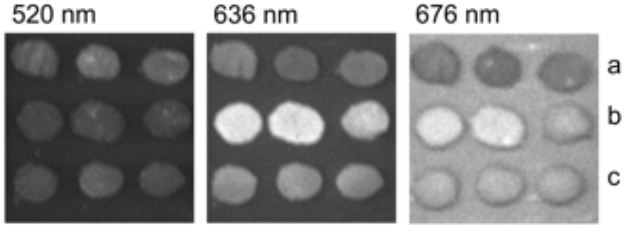

Figure 6. Normalized fluorescence responses of three brands of roast beef (a, b, c) on SS at 520, 636, and 676 nm. Note the improved contrast at 636 nm.

Conclusion

Hyperspectral images of fluorescence responses to violet (405 nm) excitation indicate that fluorescence imaging can be used to detect deli residues that might remain after cleaning and sanitation. Analyses of responses for four brands each of four cheeses and four meats suggest that good wavelengths for detection of deli residues would be 520 and 675 nm. A third wavelength that might increase the likelihood of detecting a small sub-set of the commodities tested is around 630-640 nm. These results provided encouragement for testing the use of fluorescence imaging as a tool for surveying the hygienic status of surfaces in delicatessens following normal cleaning procedures.

Conflict of Interest

There is no conflict of interest.

Acknowledgements

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