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Research Paper

Multispectral fluorescence imaging for detection of bovine faeces on Romaine lettuce and baby spinach leaves☆

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Hyperspectral fluorescence imaging with ultraviolet-A excitation was used to evaluate the feasibility of two-waveband fluorescence algorithms for the detection of bovine faecal contaminants on the abaxial and adaxial surfaces of Romaine lettuce and baby spinach leaves. Correlation analysis was used to select the most significant waveband pairs for two-band ratio and difference methods in distinguishing contaminated and uncontaminated leaf areas. For this investigation, two-band ratios using bands at 665.6 nm and 680.0 nm (F665.6/F680.0) for lettuce and at 660.8 nm and 680.0 nm (F660.8/F680.0) for spinach effectively differentiated all contamination spots applied to the lettuce and spinach leaves, respectively. The fluorescence emission peaks for the faecal matter of animals that consume green plant materials and for chlorophyll *a* occur in close proximity in the red spectral region. Consequently, a high spectral resolution would be required for multispectral imaging with these two-band ratios for online implementation to detect bovine faecal contamination on leafy greens such as Romaine lettuce and baby spinach.

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1. Introduction

In the United States, foodborne illnesses result in costs totalling approximately $152 billion annually, with problems related to raw produce accounting for $39 billion of this total. Of the 76 million cases of foodborne illness estimated to occur annually in the United States, 29.1% are related to raw produce specifically (Hoffmann, Fischbeck, Krupnick, & McWilliams, 2007; Mead et al., 1999). In 2006, an outbreak of *Escherichia coli O157:H7* due to contaminated bagged spinach from California affected 26 states (Wendel et al., 2009). In 2008, an outbreak of *Salmonella saintpaul* covered 43 states and was finally traced to contaminated Serrano and Jalapeño peppers. A recent *E. coli* outbreak in May 2010 was attributed to Romaine lettuce and affected 23 states (CDC., 2010). While the types of pathogens most likely to be associated with consumption of raw produce are known, the sources and modes of transfer of these organisms are still being researched. Animal faeces are thought to be the ultimate source of most of these organisms, and intrusion of animals into fields is thought to be an important initial vector for transfer to produce (Heaton & Jones, 2008). Cross-contamination is thought to be another important transfer vector, with about 1/3 of outbreaks due to post-harvest cross-contamination (Gerner-Smidt & Whichard, 2007). Direct identification of the inoculum sources responsible for contamination of fresh produce is usually difficult, if not impossible, because sources can include soil, contaminated irrigation water, insects, inadequately composted manure, animal/human faeces, and human handling. However, animal and human faecal matter, which represent significant sources of human pathogens such as *E. coli* O157:H7, *Salmonella* and *Cryptosporidium*, are thought to be the major sources of contamination of produce (Buck, Walcott, & Beuchat, 2003; Heaton & Jones, 2008). Consumption of fresh leafy green vegetables (e.g., lettuce, alfalfa, spinach, and sprouts), and fresh fruits contaminated by faecal matter can cause human infection (Xicohtencatl-Cort & Sanchez Chacon, 2009).

Efforts to reduce food safety risks and prevent foodborne illness include research to develop methods for detecting faecal contamination on food products. The Agricultural Research Service (ARS), USDA, in Beltsville, MD, has developed hyperspectral imaging techniques for food safety and quality assessment applications such as the detection of faecal contamination on apples (Kim, Lefcourt, Chao, et al., 2002, Kim, Lefcourt, Chen, et al., 2002; Liu, Chen, Kim, Chan, & Lefcourt, 2007) and cantaloupes (Vargas et al., 2005), and the detection of microbial contamination such as bacterial biofilms on food processing surfaces (Jun, Kim, Lee, Millner, & Chao, 2009). In particular, ARS researchers have led the development of hyperspectral fluorescence-based imaging methods and systems for detection of faecal contamination on produce. Fluorescence methods were found to be more sensitive than reflectance methods for detecting faecal contamination spots not visible or easily discernible to the naked eye, as was demonstrated by studies using aqueous dilutions of bovine faecal matter as well as thin faecal smear spots applied to apples (Kim, Lefcourt, Chen, et al., 2002; Kim, Lefcourt, Chen, & Yang, 2005). The faecal contamination spots were detected on a variety of apple colours and shapes, even with the presence of surface defects, leaves, stems, and calyxes (false positives based on reflectance or RGB imaging that lacks specificity for faecal detection) in the images. In these studies, two-waveband fluorescence image ratios (e.g., F670/F450 or F670/F550) were used for the faecal detection. In subsequent studies using an online apple sorting machine operating at a processing speed of over 3 apples per second, the simple image ratio methods demonstrated the detection of faecal contamination on apples with over 99% detection rate (Kim et al., 2007), and a multispectral imaging system was demonstrated to be capable of simultaneously inspecting for both faecal contamination and defects on apples (Kim et al., 2008). To provide comprehensive safety inspection of produce, methods to rapidly evaluate whole surfaces of produce will be needed in conjunction with the spectral imaging technology.

In this paper, hyperspectral fluorescence imaging analysis was used to determine suitable multispectral bands for detection of bovine faecal matter on two types of leafy green vegetables, Romaine lettuce and baby spinach. Faecal detection on leafy greens may present some challenges because of the relatively higher concentrations of chlorophylls found in leafy green leaves (as compared to apples) and the close proximity in red fluorescence emission characteristics between the animal faecal matter and chlorophyll *a* in green leaves. Furthermore, the chlorophyll *a* content of spinach is approximately 3 times higher than that of Romaine lettuce (Aguero, Barg, Yommi, Camelo, & Roura, 2008; Rapsch & Ascaso, 1985), and spinach leaves exhibit a relatively homogeneous surface while the veined leaves of Romaine lettuce exhibit greater variegation. These leaf characteristics may complicate the detection of faecal contamination by spectral imaging methods. The objectives of this study were to determine the most significant two wavelengths and develop multispectral imaging algorithms to detect faecal contamination on leafy greens, ultimately for use in online inspection applications at produce processing plants.

2. Materials and methods

2.1. *Plant material and dairy manure preparations*

Romaine lettuce (*Lactua sativa*) and baby spinach (*Spinacia oleracea* L.) were purchased from a local market. The outermost leaves of the lettuce head were aseptically removed and two or three inner leaves were taken for the experiments. The leaves were washed under running water for 30 s, and dried at room temperature. Freshly deposited faeces from Holstein cows were collected at the USDA-ARS Dairy Operations Unit in Beltsville, MD, and stored in a frozen state at −4 °C prior to their use in this study. Faecal contamination treatments were applied to a total of 40 leaves each of Romaine lettuce and baby spinach—for each species, 20 on adaxial surfaces and 20 on abaxial surfaces to develop methods that work on both surfaces of leaf samples. Six faecal smear spots on each leaf surface, arranged with a column of three spots to each side of the leaf mid-vein, were created using a micro spatula. Each contamination spot was approximately 5 mm in diameter.