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Hyperspectral imaging for the detection of microbial spoilage of mushrooms

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ABSTRACT

Brown blotch, caused by pathogenic *Pseudomonas tolaasii*, is the most problematic bacterial disease in *Agaricus bisporus* mushrooms; it reduces their consumer appeal in the market place, thus generating important economical losses worldwide. The mushroom industry is in need of fast and accurate evaluation methodologies to ensure that only high quality produce reaches the market. Hyperspectral imaging (HSI) is a non-destructive technique that combines imaging and spectroscopy to obtain spatial and spectral information from an object. The aim of this study was to investigate the potential of Vis-NIR HSI to identify microbiological damage in mushrooms and to discriminate it from mechanical damage. Hyperspectral images of mushrooms subjected to i) no treatment, ii) microbiological spoilage and iii) mechanical damage were taken during storage and spectra representing each of the classes were selected. Partial least squares-discriminant analysis (PLS-DA) was carried out in two steps: i) discrimination between undamaged and damaged mushrooms and ii) discrimination between damage sources (i.e. microbiological or mechanical). The models were applied at a pixel level and a decision tree was used to classify mushrooms into one of the aforementioned classes. A correct classification of >95% was achieved. This was the first reported study to employ HSI for the detection of damage of bacterial origin in horticultural products. The industry could incorporate the knowledge gained in this study towards the development of a HSI sensor to detect and classify mushroom damage of microbial and mechanical origin, enabling the rapid and automated identification of mushrooms of reduced marketability.

Keywords: mushrooms; brown blotch; Pseudomonas tolaasii; mechanical damage; vis-NIR hyperspectral imaging; PLS-DA.

INTRODUCTION

Cultivated mushrooms are susceptible to a variety of pests and diseases. *Pseudomonas tolaasii* (*P. tolaasii*) is the causal agent of brown blotch (also known as bacterial blotch) disease [1] and the most important pathogenic bacterium of *Agaricus bisporus* [2] This disease has been detected and described worldwide and affects not only the button mushroom market but the mushroom market in general [3]. The colonisation of mushroom caps by *P. tolaasii* results in the appearance of unappealing brown spots on the mushroom cap and stipe. Lesions are slightly concave blemishes, sometimes small, round or spreading in many directions [4]. When the damage is more intense, the spots are darker and sunken. Browning affects only the external layers of the cap tissue and is restricted to 2-3 mm below the surface of the cap.

The mushroom industry is in need of objective evaluation methodologies to ensure that only high quality produce reaches the market [5]. Studies in the field of brown blotch detection include the work of Vízányó and Felföldi [6], who tested the potential of a machine vision system to recognise and identify brown blotch and ginger blotch diseases, both of which cause discolouration in mushroom caps. HSI is a rapid and non-destructive technology that has recently emerged as a powerful alternative to conventional imaging for food analysis [7]. Hyperspectral images are composed of hundreds of contiguous wavebands for each spatial position of an object. Consequently, each pixel in a hyperspectral image contains the spectrum of that specific position. The large quantities of highly correlated data contained in a hypercube are well suited to analysis by dimension reduction approaches such as principal components analysis (PCA) and partial least

squares- discriminant analysis (PLS-DA) [8]. PLS-DA can also be applied to develop qualitative models for supervised classification between various sample classes.

HSI has been applied at various levels in the assessment of safety and quality of food, including constituent analysis, quality evaluation and detection of contaminants and defects [7]. Additionally, a number of researchers have reported the potential of HSI for identification of microorganisms of concern in food [9-10]. Recent advances in the detection of skin damage of other products include work by Ariana *et al.* [11] with cucumbers and Nicolai *et al.* [12] and ElMasry *et al.* [13] with apples. As regards damage of microbial origin, Gómez-Sanchis *et al.* [14] proposed a HSI system for the early detection of rot caused by *Penicillium digitatum* (fungi) in mandarins.

While evidence from the literature points to its feasibility, to the authors' knowledge, HSI has not been used to detect damage of bacterial origin in horticultural products. The objective of this study was to investigate the potential application of Vis-NIR HSI for brown blotch identification on mushroom caps and for its discrimination from mechanical damage injuries.

MATERIALS & METHODS

Mushroom supply and damage

Agaricus bisporus mushrooms were grown in plastic bags and tunnels in Kinsealy Teagasc Research Centre (Kinsealy, Ireland) following common practice in the mushroom industry. Samples were placed in a metal grid and carefully delivered to the laboratory in purpose-built containers, to minimise damage during transport. Two flushes of mushrooms were picked: training set and test set.

For each set of mushrooms ($n_{\text{train}} = 144$ and $n_{\text{test}} = 108$), samples were divided in 3 groups (undamaged (U), mechanically damaged (MD) and *P. tolaasii* inoculated mushroom (PT)) of equal size ($n_{\text{train},i} = 48$ and $n_{\text{test},i} = 36$, where $i = \text{U, MD, PT}$).

Each mushroom class was treated as follows:

- **U:** No treatment.
- **MD:** samples were subjected to vibrational bruising to simulate crop handling and transport. Samples were stored for 24 h prior to imaging.
- **PT:** samples were obtained by inoculating a solution of pathogenic *P. tolaasii* onto each clean cap. Samples were stored for 48 h prior to imaging, to encourage appearance of brown blotch symptoms on the mushroom caps.

A total number of 252 mushrooms were used in this experiment.

Hyperspectral imaging

Hyperspectral images were obtained using a pushbroom line-scanning HSI instrument (DV Optics Ltd, Padua, Italy) within the wavelength range of 445-945 nm. Reflectance calibration was carried out prior to mushroom image acquisition in order to account for the background spectral response of the instrument. HSI images of U mushrooms were acquired on day 0 of the experiment. MD mushrooms were scanned after 24 h of storage. PT mushroom images were taken after 48 h of storage. Data were recorded in units of reflectance and saved in ENVI header format using the acquisition software.

Image processing

For each mushroom hyperspectral image, 175 characteristic (i.e. U, MD or PT, depending on mushroom class) regions of interest (ROI) were selected from the central region of the mushroom cap,. Selecting spectra from analogous surface areas in all the mushrooms aimed at minimising the scaling differences caused by mushroom surface curvature [15]. The average reflectance spectrum of each ROI was obtained by averaging the pixel spectra of the region. Spectral data of each mushroom set were used to build two-dimensional matrices, where each row represented the spectrum of one ROI.

The experiment was repeated twice, making two independent sample sets for model training and testing. Training set matrices contained 8400 spectra and test set matrices contained 6300 spectra.

Partial least squares- discriminant analysis (PLS-DA)

Partial least-squares discriminant analysis was applied to the training set matrices ($n=8400$) using MATLAB 7.0 (The Math Works, Inc. USA). The aim was to build models that would enable maximum separation of sample spectra into different classes depending on their physical condition. A two step model approach was taken: one model (namely "U/Dam" model) was developed to discriminate between undamaged (U) and

damaged (Dam) spectra and another model (namely “MD/PT” model) was built to discriminate between the two classes of Dam, i.e. mechanical (MD) and microbiological (PT).

The models were also applied to the test set matrices, which were used as an independent set of sample spectra. Performance of the classification models was evaluated on the basis of their sensitivity (number of spectra of a given type correctly classified as that type) and specificity (number of spectra not of a given type correctly classified as not of that type) on the training and test sets.

Prediction maps

An important feature of hyperspectral imaging is the ability to map the distribution of components/attributes on samples. In this case, developed PLS-DA models were applied to entire hypercubes of mushrooms to form two dimensional prediction images where the damage class of each pixel as predicted by the PLS-DA models was represented by its intensity. The concatenation of the three binary maps led to false colour maps where U, MD and PT classified pixels were represented in green, red and blue, respectively.

Mushroom classification

Based on the percentage of pixels of each damage class on the prediction map, a decision tree (shown in Figure 1) was used to allocate each mushroom to one of the three mushroom classes. Sensitivity and specificity of the classification procedure were computed after the application of the decision tree to all of the mushroom hypercubes.

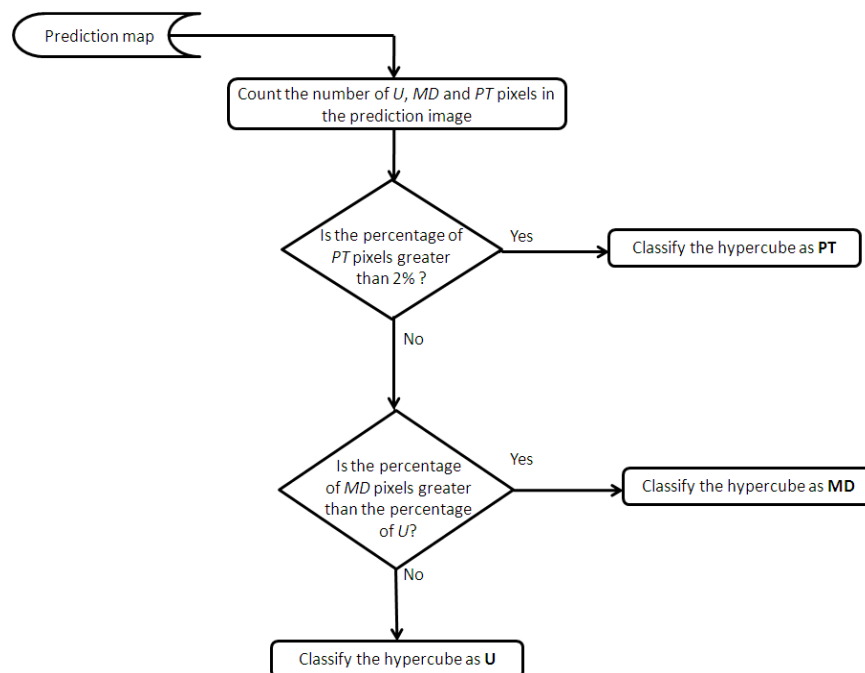


Figure 1 Decision tree for mushroom hypercube classification. U = undamaged; MD = mechanically damaged and PT = *P.tolaasii* inoculated.

RESULTS & DISCUSSION

RGB images

Figure 2 shows representative colour images of the three mushroom classes under investigation in this study. Mushrooms labelled as U (Figure 2a) were white in general appearance, although some of them showed some signs of natural discolouration caused by common picking and transport practice. By day one of storage, MD samples (Figure 2b) exhibited uniform browning over the entire mushroom surface. By day two of storage, *P. tolaasii* had colonised the cap of most PT mushrooms (Figure 2c), which exhibited slightly concave brown-coloured spots, the typical symptoms of brown blotch disease.

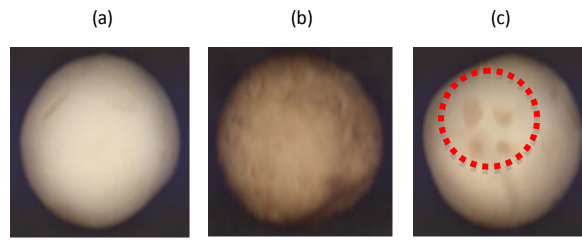


Figure 2 RGB images of the mushrooms under investigation: (a) undamaged (U); (b) mechanically damaged (MD) and (c) *P.tolaasii* inoculated (PT).

Spectra

Mean spectra of the various spectra classes are shown in Figure 3. Signal intensity and shape differences between U and MD spectra were remarkable. The mean MD spectrum exhibited lower reflectance values over the entire spectral region, as expected after bruising had led to loss of whiteness of the caps. The greatest differences in shape between MD and U spectra arose in the 600-800 nm region, where the mean U spectrum exhibited broader features than the mean MD spectrum. Broad spectra in the visible-near infrared wavelength range are characteristic of undamaged mushrooms, corresponding to their white appearance. The spectral differences mentioned above could be related to the formation of brown pigments, mainly melanins, which derive from enzyme-catalysed oxidation products called quinones. The mean PT spectrum appeared to be more similar in shape to the mean MD spectrum, although its slope was not as linear as MDs was in the 600-800 nm region.

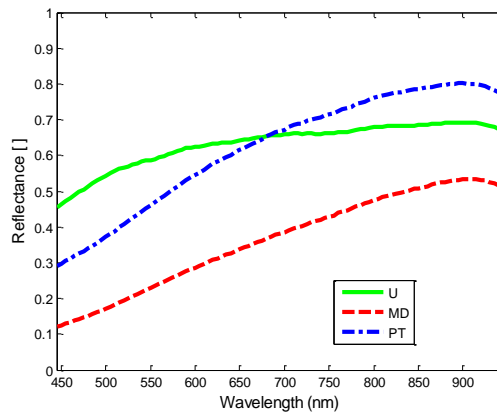


Figure 3 Mean spectra of the mushroom classes under investigation: U = undamaged; MD = mechanically damaged and PT = *P.tolaasii* inoculated.

PLS-DA analysis

Performance statistics of the selected models are shown in Table 1. When the models were applied to the training set of spectra, almost perfect classification was achieved in the case of the U/Dam model (sensitivity = 0.997 and specificity = 1.000). Similarly, almost all of the Dam spectra were classified as such and none or only a few U were misclassified as Dam. When the MD/PT model was applied to the damaged spectra, the sensitivity was 0.988, whereas the specificity was 0.983. These results showed that almost all of the spectra of the mushrooms that had been inoculated with *P. tolaasii* were classified correctly and only a few or none of the spectra of the MD samples were misclassified as PT.

When the models were applied to the test set of spectra, the sensitivity of the U/Dam model was lower (sensitivity = 0.832) but still none of the U spectra were misclassified as Dam (specificity = 1.000). When the MD/PT model was applied to the damaged spectra of the test set, a smaller percentage of raw PT spectra were classified correctly (sensitivity = 0.661) but almost none of the MD spectra were misclassified as PT (specificity = 0.984).

Table 1 Performance statistics at spectra level of the PLS-DA models built on reflectance spectra.

Model	TRAINING SET		TEST SET	
	Sensitivity	Specificity	Sensitivity	Specificity
U/Dam	0.997	1	0.832	1
MD/PT	0.988	0.983	0.661	0.984

2

Prediction maps

Figure 4 shows false colour prediction maps of (a) U, (b) MD and (c) PT mushroom samples as a result of the application PLS-DA models to the data hypercubes. In the example shown, neither the map of the U mushroom nor the central region of the prediction of the MD mushroom showed misclassification, whereas edge pixels of the latter were misclassified as U. Considering that all the spectra selected for model building belonged to central regions of the mushrooms, this misclassification could be related to the inability of the models to account for spectral differences due to mushroom surface curvature. A few pixels were misclassified in the prediction map of the PT mushroom, where some pixels were classified as MD.

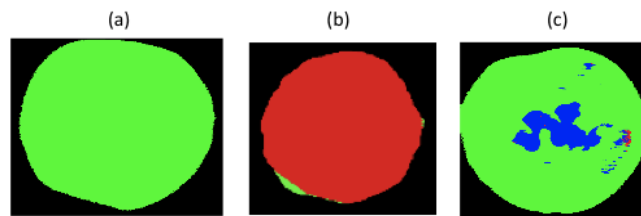


Figure 4 Prediction images of mushrooms belonging to the test set: (a) undamaged (U); (b) mechanically damaged (MD) and (c) *P.tolaasii* inoculated.

Mushroom classification

The application of PLS-DA models to the totality of entire hypercubes led to the performance statistics shown in Table 2. For the training set mushrooms, both the sensitivity and the specificity of the U/Dam_raw model were 1, with no misclassification at all. For the same samples, the sensitivity of the MD/PT_raw model was 1 and its specificity was 0.98. Only 1 out of 48 MD mushroom was misclassified as a PT mushroom. The models performed quite similarly for the mushroom hypercubes of the test set: for the U/Dam_raw model, sensitivity = 0.97 and specificity = 1. Only 2 out of 72 Dam mushrooms were misclassified as U, and none of the U was misclassified as Dam. For the MD/PT_raw model, sensitivity = 0.944 (only 2 out of 36 PT mushrooms were not classified as such) and specificity = 0.97 (only 1 MD mushroom was misclassified as being PT).

Table 2 Performance statistics at a pixel level of the PLS-DA models built on reflectance spectra.

Model	TRAINING SET		TEST SET	
	Sensitivity	Specificity	Sensitivity	Specificity
U/Dam	1	1	0.972	1
MD/PT	1	0.979	0.944	0.972

These results show the models performed well when applied at a pixel level and could be the first step towards the development of a HSI sensor that would classify independent sets of mushrooms with high levels of accuracy. Overall, the correct classification of the models presented in this paper is higher than the classification of the algorithms by Vízványó and Felföldi [15], which correctly classified 81% of the diseased areas of test mushrooms using conventional computer imaging. While the algorithms presented in the aforementioned paper discriminated diseased spots from healthy senescent mushroom parts, the models

developed in this paper discriminate microbial spoilage from both undamaged and mechanically damaged samples. The correct discrimination between PT and MD mushrooms ensure no misclassification of samples whose colour analysis might be similar and hence avoid “false positives”.

CONCLUSION

This was the first reported study to employ HSI for the detection of damage of bacterial origin in horticultural products. The results demonstrate the potential use of hyperspectral imaging as an automated tool for detection of brown blotched mushrooms and for their discrimination from mechanically damaged mushrooms. Knowledge gained in this research using HSI is being employed to develop of simpler sensors which detect and classify mushroom damage of different sources. Such a system will aid the industry in increasing quality control standards by correctly identifying low quality produce.

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