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EARLY DETECTION OF COBWEB DISEASE INFECTION ON *AGARICUS BISPORUS* SPOROCARPS USING HYPERSPECTRAL IMAGING

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From the nineteen-nineties, cobweb disease caused serious losses for the mushroom sector in Europe, in the USA, and in Australia (Fletcher & Gaze, 2008), so it is one of the most notable fungal infections of cultivated white button mushroom (*Agaricus bisporus*). The aim of this study was to identify cobweb disease (*Cladobortyum dendroides*) caused cap spotting and brownish rot on the mushroom sporocarp, and to find a proper discrimination method in the case of this infection.

Fruiting body samples were divided into 4 groups, a control one and three others treated with different chemicals that are tested against fungal infections. The groups were subdivided into 2 portions and the first was infected with cobweb disease. Images of the caps were recorded and their hyperspectral images were acquired in the wavelength range of 900–1700 nm.

On the hyperspectral images infected and healthy areas were selected, on these average spectra differences were found around the known water peaks (1200 and 1450 nm). The spatial distribution of the water content can be used for the detection of the spoilage, because the infected areas showed different reflection values at these water absorption peaks.

Support Vector Machine method was applied successfully to discriminate between the infected and control groups and Monte Carlo cross-validation was carried out.

Keywords: mushroom, *Cladobotryum*, *Dactylium*, HSI, SVM, MCCV

The chemical composition of mushrooms is beneficial for health (because of protein constitution, high polysaccharide content, Vitamin B and D_2 content), so they became more popular amongst customers in the last years (Wani et al., 2010). White button mushroom *(A. bisporus)* is one of the most widely cultivated mushrooms in the world, around one third of the total global mushroom production is dedicated to the production of *A. bisporus* species (Geösel et al., 2011).

Button mushroom can be attacked by some viral or bacterial diseases, microscopic fungi and insects, which cause great losses for the mushroom sector. Cobweb disease is a well-known fungal infection, caused by two subspecies of *Cladobotryum dendroides* (synonym of *Dactylium dendroides* Fletcher & Gaze, 2008*;* Mycobank, 2014). The name of this disease refers to its most typical symptom, when the cobweb-like mycelial growth appears on the surface of the fruiting body or the casing soil. The fruiting body becomes light brown and

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shrinkage is observed. Two types of spots appear on the cap: the first type is dark brown with indefinite contour; the second type is light brown with round contour (GYŐRFI, 2010).

Hyperspectral imaging (HSI) is a promising, rapid, and non-destructive technology; it was first used for airborne remote sensing applications. In the push-broom setup, a spectrograph disperses light into a spectrum, mapping all points of the examined line into the rectangle area of sensor matrix (FIRTHA, 2007). HSI can provide both spatial and spectral information from the sample, therefore this method can be very useful if the distribution of a component or other feature is not homogeneous in the sample. Accordingly this technique became very popular in food quality measurements (GOWEN et al., 2007; WU & SUN, 2013).

Previous studies at Dublin Institute of Technology investigated champignon with hyperspectral imaging. GOWEN and co-workers (2008a) measured quality in terms of moisture content, colour, and texture, while TAGHIZADEH and co-workers (2009) predicted moisture content with Partial Least Squares Regression (PLSR) models, using a pushbroom system operating in the wavelength range of 400–1000 nm, having a high performance CCD camera (BASLER vision technologies, Germany) and a spectrograph (Specim V10E, Finland). GOWEN and co-workers detected mechanical (2008b) and freeze damage (2009) using Principal Component Analysis (PCA) and Linear Discriminant Analysis (LDA) methods, hyperspectral images were obtained using a pushbroom line-scanning HSI instrument, operating in the wavelength range of 400–1000 nm. TAGHIZADEH and co-workers (2011) applied hyperspectral imaging (HSI) and developed Partial Least Squares Discriminant Analysis (PLS-DA) models to discriminate between casing soil, enzymatic browning, and undamaged tissue on mushroom surfaces. GASTON and co-workers (2011) investigated the potential of Vis-NIR HSI to detect microbial spoilage, namely *Pseudomonas tolaasii* (brown blotch) and to discriminate it from mechanical damage.

The objective of the present study was to investigate the potential of hyperspectral imaging method for the detection of the cobweb infection on the mushroom sporocarps, since the symptoms appear as inhomogeneity of the surface and the differences of the spectra are observable even in early stages.

1. Materials and methods

1.1. Mushroom samples

White button mushroom *(Agaricus bisporus)* was cultivated and harvested under controlled conditions at the Corvinus University of Budapest. Phase III compost and 5 cm Hungarian non-treated casing were the used. The cultivation parameters (compost temperature, humidity, CO_2, irrigation) were same in each treatment. Mushroom samples were split into 4 groups: three groups were treated with different materials used in the cultivation practice (active substances: 1. natamycin, 2. prochloraz-Mn, 3. *Bacillus subtilis*) and one group was not treated. The four groups were divided into two portions and one portion of the samples was directly infected at casing with cobweb disease *(Cladobotrynum dendroides)* in a concentration of 10^6 CFU m^{-2}. The typical symptoms (FLETCHER & GAZE, 2008) were observable by visual perception at the end of first flush that confirms the successful infection. The number of the samples in the 8 groups varied, there were 61 infected and 39 not infected samples (untreated: 29, natamycin: 26, *Bacillus subtilis*: 23, prochloraz-Mn: 22 samples). Mushroom sporocarp samples from first flush were stored in a refrigerator for 11 days.

1.2. Hyperspectral imaging

After the harvest, images of the mushroom caps were recorded by a digital camera (Canon EOS 450D). The hyperspectral images were recorded with 45/0 geometry on every day of the storage using a pushbroom HSI instrument (Headwall Photonics XEVA-1648 XC134: Specim spectrograph, Xeneth InGaAs 14 bit sensor having 256×320 resolution) within the wavelength range of 900–1700 nm. The setup of optics finally resulted 5 nm spectral and 0.475 mm spatial resolutions.

The image processing system and the sensor were controlled by Argus hyperspectral software (FIRTHA et al., 2012). Before all measurement series, the signals of dark and bright standards (NCS 0300) were measured to calculate reflectance (spectral reflection factor) of samples.

1.3. Image segmentation and pre-processing of spectra

During the training phase, infected and healthy areas (regions of interest) were selected manually on the hyperspectral images using CuBrowser MATLAB software (FIRTHA & ÉDER, 2012) and the average spectra of areas were saved.

To reduce the disturbing effects of non-homogeneous illumination, a simple normalization algorithm (the average of the intensities on the whole spectrum was subtracted from the single intensity values) and a Savitzky-Golay smoothing (polynomial order:3, window size:9) were carried out on the spectra.

1.4. Statistical evaluation

Support Vector Machine (SVM) is a classifier method that performs classification tasks by constructing hyperplanes in a multidimensional space that separates cases of different class labels. To construct an optimal hyperplane, SVM employs an iterative training algorithm, which is used to minimize an error function (STATSOFT, INC., 2013).

The model was built using RStudio version 0.97.336 software. The data of the infected and control samples were separated group by group, using Support Vector Machine method (e1071 package, developed at Vienna University of Technology).

Monte Carlo cross-validation (MCCV) method (XU & LIANG, 2001) was carried out with 20% of the samples as validation set (and 80% as training sample set) without optimisation. This process was repeated 10^3 times.

2. Results and discussion

Figure 1 shows that there are real spectral differences between the control and the infected samples. Around the known water peaks (1200 and 1450 nm) high absorption is noticeable. Since at 1450 nm the infected spots show higher reflection (meaning lower absorption), the moisture content of these infected spots may be different.

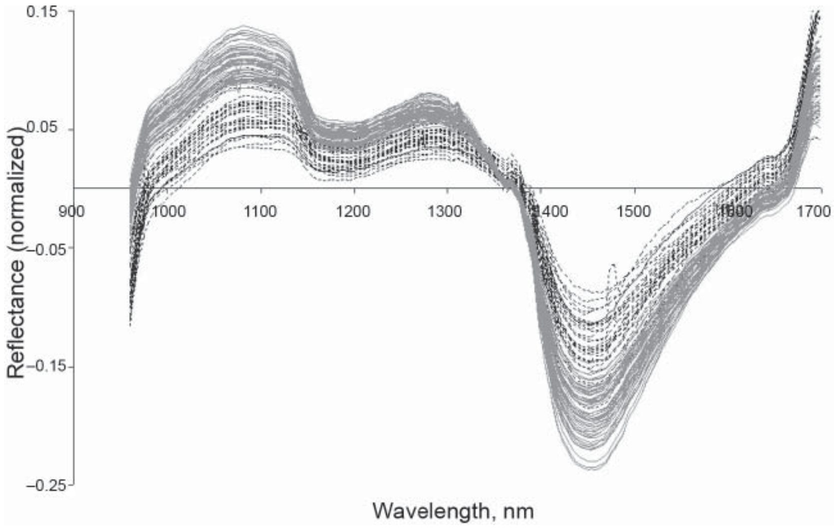

Fig. 1. Measured spectra on the first day of an experiment
(black dotted: *Dactylium* infected spot; grey continuous: control)

At the other characteristic water absorption peak, the difference between the spectra is smaller, therefore after the normalization the infected spots show lower absorption.

The most conspicuous differences of the two types of spectra can be seen around 1450 nm and 1080 nm, comparing these two reflection factors seems to be characteristic to the infection. The presence of the infection can be recognised mainly because of the non-homogeneity of these reflection values and the distribution of the moisture content.

The differences of the moisture content of the tissue can be shown on the slice of the hypercube at 1450 nm (cross-section), so the intensity of the reflection refers to the water content. Figure 2 shows the changes of a natamycin treated sample around 1450 nm wavelength during 11 days of storage. On the normalized cross-section of the hyperspectral images, the symptom of the disease appears as a light spot (red and black marks), mechanical damaged regions seem to be dark areas (blue and green marks), so by this method the state of the tissue could be visualized, and the cause of the damage is recognizable.

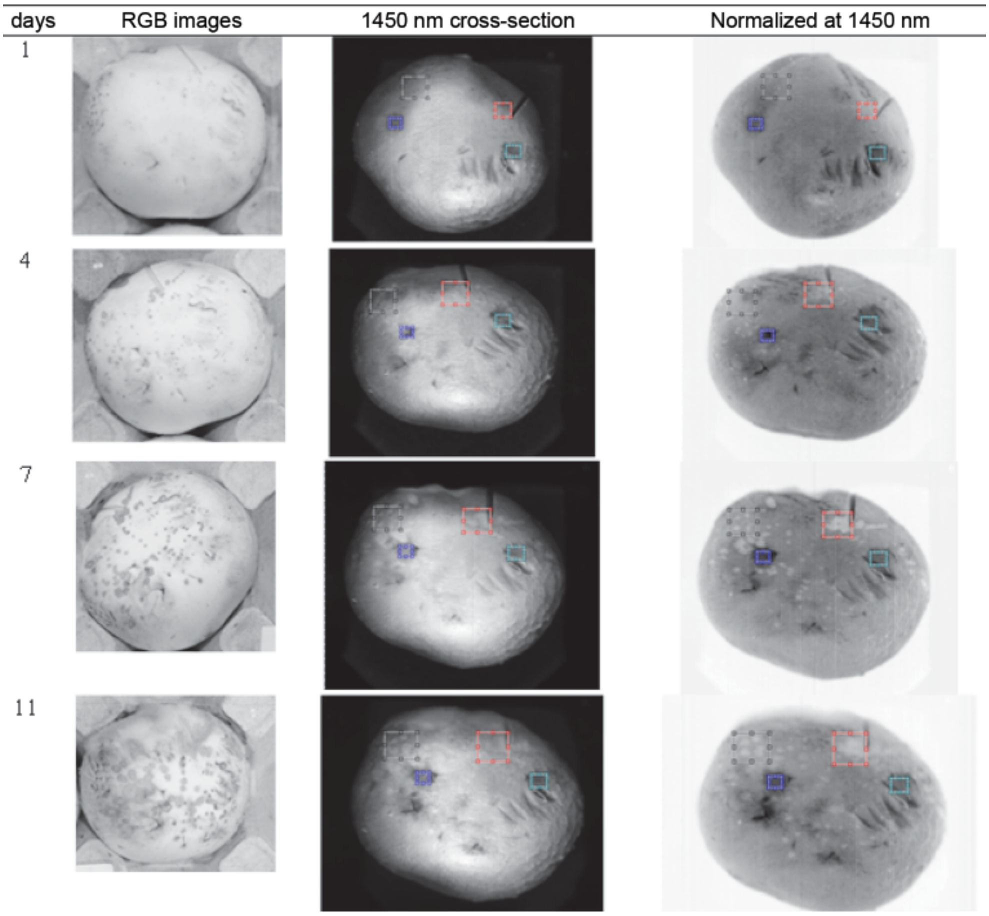

Fig. 2. Images of a natamycin treated sample (□ black, □ red: *Dactylium* infected spots; □ blue, □ green: mechanically damaged regions)

The results of the classification with SVM method are shown in Table 1. The classification of the samples was successful. The ratio of the correctly classified samples into not treated, natamycin treated, prochloraz-Mn treated and *B. subtilis* treated groups were 100%, 77.27%, 89.23%, and 92.33%, respectively.

During the validation, this rate decreased slightly: in not treated, natamycin treated, prochloraz-Mn treated and *B. subtilis* treated groups were around 98.5%, 78%, 88%, and 91%, respectively (Table 2).

These results are promising even in comparison with the work of Gaston and co-workers (2011), however, they studied the spectra of *Ps. tolaasii* infected and mechanical damaged mushroom caps in the wavelength range of 445–945 nm. In their study the ratio of correctly classified samples was 98.8% in the case of training set and 66.1% by the test set.

Table 1. Results of SVM classification (a.: not-treated, b.: treated with prochloraz-Mn, c.: treated with natamycin, d.: treated with *B. subtilis*)

a.

Not treated		
	True	
Predicted	*Dactylium*	Control
Dactylium	100%	0%
Control	0%	100%
Correct classification	100%	

b.

Prochloraz-Mn		
	True	
Predicted	*Dactylium*	Control
Dactylium	95.42%	4.58%
Control	16.96%	83.04%
Correct classification	89.23%	

c.

Natamycin		
	True	
Predicted	*Dactylium*	Control
Dactylium	60.13%	39.87%
Control	5.60%	94.40%
Correct classification	77.27%	

d.

B. subtilis		
	True	
Predicted	*Dactylium*	Control
Dactylium	88.89%	11.11%
Control	4.24%	95.76%
Correct classification	92.33%	

Table 2. Results of Monte Carlo cross-validation

	Average ratio of correctly classified samples
Not treated	98.5%
Prochloraz-Mn	78%
Natamycin	88%
B. subtilis	91%

3. Conclusions

The quality monitoring of white button mushroom with hyperspectral imaging is a novel, exciting field of application (only a few studies can be mentioned). Previous research about the detection of *Ps. tolaasii w*as successful (Gaston et al., 2011), however, Gaston (2010) could not reach sufficient detection of Mushroom Virus X.

According to the results of this study, the normalized relative reflectance showed differences between the *Dactylium* infected and the control samples. On the normalized 1454 nm cross-section of hypercube the spoilage can be recognized and its appearance is different from the mechanical damage.

The cobweb disease infected samples were successfully separated with SVM method, the ratio of the correctly classified samples was over 80% in every group and higher than 75% in the validation process.

At this point of the research we can aim at further possibilities. A potential way can be the improvement of the detection at those stages, when visual perception or a digital camera cannot observe it. Finally, a multispectral classification system should be developed for a cost-effective and preferably automated (industrial) application.

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