Optical inspection and monitoring of moisture content in *Pleurotus eryngii* during storage life by refrigeration

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Abstract

Mushrooms have a very high moisture content and a good source of vitamins and minerals. To maintain the moisture content of mushroom is of utmost importance to maintain the quality of the mushroom during storage life. To have a non-destructive and non-invasive methods of moisture content inspection would aid the maintenance of mushroom to great lengths. We proposed a novel application of using optical coherence tomography as an optical inspection tool and also to monitor the moisture content in mushrooms to ensure in maintaining the freshness of mushrooms. In this study, we have used *Pleurotus eryngii* mushroom samples to inspect the moisture content and monitor them for consecutive days to study the structural changes in them. A-scan (axial depth scan) analysis was used to moisture content analysis in the mushroom body and thereby, supporting the experiment results.

Keywords: Optical coherence tomography, optical inspection and monitoring, *Pleurotus eryngii*, moisture content.

INTRODUCTION

Pleurotus eryngii (King oyster muchroom) is widely consumed throughout the world population. *P. eryngii* comes under the oyster mushroom family, it is known to have antioxidative properties [1, 2]. Its culinary qualities and longest shelf life in comparison to other oyster mushroom [3] makes it one among the important food harvest in many countries. It can be easily and successfully being cultivated on wheat and rice straw, cotton waste and sawdust [4]. Color and texture of mushrooms are of major priority to customers which are affected by physico-chemical treatments to mushrooms [5]. Biophysical properties of mushroom can be affected during cooling down [6]. Many methods have been taken and implemented to overcome these issues. One such method is by using moisture absorbers for packaging [7]. But it is essential to know the state of product during its refrigeration to avoid any wastage of harvest. Thus, a non-invasive and non-destructive inspection technique can help in such conditions and avoid wastage of mushrooms by moisture loss.

Many non-destructive plant inspection studies have been reported that uses computed X-ray tomography, positron emission tomography (PET), magnetic resonance imaging (MRI), and optical coherence tomography (OCT) [8-13]. Amidst these imaging techniques, OCT is shown to be a powerful imaging technique that can potentially obtain two dimensional (2D) and three dimensional (3D) images with high resolution [14, 15]. The capability of offering cross-sectional images without the need for sectioning, with high resolution, fast acquisition speed, and high sensitivity are the notable features of OCT. as a result of the above mentioned features, OCT has been broadly used in various biomedical fields such as ophthalmology [16, 17], dermatology [18], otolaryngology [19], and industrial applications [20, 21]. OCT has also been used for water absorption in plant tissues and in seeds [22]. So far, OCT as a tool for moisture level monitoring in fungus during storage life has not been reported.

The intent of this study was to evaluate OCT as an inspection and monitoring technique for moisture content evaluation in mushroom and its relative applications in the field of agronomical studies. The morphological changes in mushroom as the moisture content reduced in time was studied. With the help of A-scan (axial depth scan) analysis the obtained results were supported.

MATERIALS AND METHOD

Mushroom sample preparation

Freshly harvested *P. eryngii* samples were obtained from the fields in South Korea and refrigerated. The fully mature samples were selected for experiments. The selected samples were carefully washed with distilled water and labeled for identification. The refrigerator temperature was maintained to -1 to 3 ℃. As mushrooms have very high moisture content, a relative humidity was maintained to around 95%. The region to be monitored on mushroom body, that is on the pileus (cap) and the stipe (stem) region, which is rich in moisture content is marked with markers in such a way that there were no damages inflicted to the samples during the process. After every

experimental session, the mushrooms were immediately refrigerated.

OCT for optical inspection and monitoring

Figure 1 shows the schematic representation of the swept source optical coherence tomography (SS-OCT) used for the optical inspection and monitoring of the *P. eryngii* specimens. The custom-built SS-OCT has a broad bandwidth swept source laser (AXP50125-6, Axsun Technology, USA) of center wavelength of 1310 nm and bandwidth with full width half maximum (FWHM) of 110 nm. The sweeping rate of the swept source is 50 kHz, with an output power of 20 mW. With the help of an 80:20 ratio optical coupler (OCT-310C32C13, Gooch & Housego PLC, UK) the laser output power was split, and the 20 percent of the coupler output was connected to a circulator (CIR-1310-50-APC, Thorlabs Inc., USA) which was connected to the reference arm setup. The reference arm comprises of a collimator, a focusing lens, and a highly reflective mirror. Furthermore, the 80 percent of the optical power from the coupler is connected to circulator, which was then connected to a sample arm setup. The sample arm consisted of collimator, galvanometer scanners, and a focusing lens which focuses the scanning beam from the galvanometer scanners on to the sample surface. The backscattered light from the sample arm and reference arm which is directed by their respective circulators output arms are then connected to an optical coupler of ratio 50:50 (OCT-310K32C13, Gooch & Housego PLC, UK). The interference signal from the output end of the 50:50 ratio coupler arms are connected to the positive and the negative ports of a balanced detector (PDB430C, Thorlabs Inc., USA). The output signal of the detector is digitized with the help of a digitizer (ATS9462, Alazar Technologies Inc., Canada).

The built system had an axial resolution of 6.8 µm and lateral resolution of 14.6 µm. A software-based data-processing technique was designed and implemented for the two-dimensional (2D) OCT image acquisition and image construction. 500 successive axial depth scans (A-scan) one next to the other positions on the sample surface were scanned and the obtained OCT images were combined in real-time to construct one 2D OCT image. With the help of the galvanometer scanners successive positions on the sample surface was scanned to obtain 2D images of desired area to make a three-dimensional (3D) volumetric image. A volume rendering software was used in post processing to construct a 3D volumetric image of the scanned sample surface (All 3D scan was taken with in a scan area of 5 x 5 mm). Wavenumber linearization method was employed to compensate distortion due to point spread function (PSF) and to increase signal-to-noise ratio [23, 24].
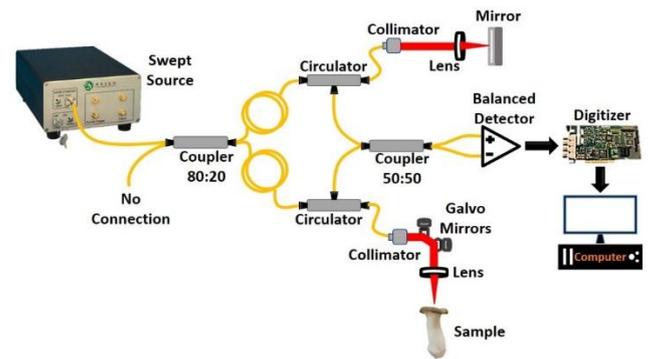

Figure 1: The schematic diagram of the SS-OCT for optical inspection and monitoring of *P. eryngi*.

A-scan analysis algorithm

To analyze the obtained OCT images, a Matlab based software program was developed and utilized for intensity peak detection in depth direction of the 2D OCT images. The executed program searches for intensity peaks which is given within a desired window size (for example, 10 A-scans). After which, the algorithm sequentially detects for maximum intensity of the A-scan signals. Followed by rearrangement of all the peak positions of A-scans within the window size while matching peak intensity index in the A-scans to flatten the image. Also, the index positions respective to high intensity are rearranged and matched linearly to get a flattened plot. Rearrangement is executed in such a way that the first intensity peaks was retrieved from every A-line of a 2D image and plotted at the beginning of the A-scan plot. It is to be noted that the absent of intensity peaks in plot may be due to presence of air or other structures of the sample represented in the image which are lesser than the detectable resolution of the OCT system. Finally, all the rearranged and flattened A-scan lines were summed up and averaged, so as to obtain an averaged A-scan profile. Then, the obtained A-scan intensity profiles were divided by the maximum value to obtain a normalized A-scan intensity plot for the desired window size of the 2D OCT image.

RESULTS AND DISCUSSION

Figure 2 shows a *P. eryngii* specimen sample, from top view as in A1 and from side view as shown in B1 images. Also, the solid red square boxes marked in A1 and B1 are the scanned region on sample surface by the SS-OCT system. In figure 2, A2 and B2 are the 2D SS-OCT images. For monitoring of moisture content in the *P. eryngii* mushroom samples, the pileus (cap) and the stipe (stem) region was chosen. In A2, the outermost layer pileipellis and the moisture rich cap trama is seen. Also, in A3, where the stem region was scanned the outermost stipitipellis layer and the moisture filled stipe trama can be observed. In order to monitor on the exact same region on consecutive days, the surface of the samples was marked

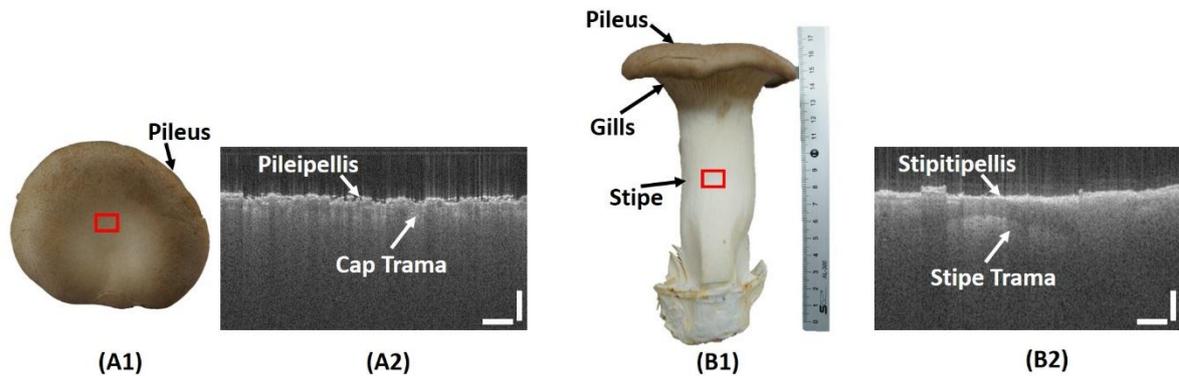carefully without causing any damage to the specimen.

Figure 2: Mushroom sample photographs along with 2D OCT images. A1 and B1 are the top view and side view photographs of the same mushroom sample respectively. Also, A2 and B2 are the OCT images scanned on the sample surface as shown in A1 and B1 respectively. The solid red box regions indicate the scanned region. The scale bar is 500 µm.

Figure 3 shows the monitoring images of the *P. eryngii* sample on consecutive days. The monitoring period of samples were carried out for 15 consecutive days, that is until the optimal expected changes were seen in sample. In figure 3, images A1-A4 represents the monitoring images of the same *P. eryngii* sample on the pileus (cap) surface. A1, A2, A3, and A4 represents the 2D OCT monitoring images of the sample taken on day 1, day 5, day 10, and on day 15. Similarly figure 3, images B1-B4 represents the monitoring images of the same *P. eryngii* sample on the stipe (stem) surface. Furthermore, B1, B2, B3 and B4 represents the 2D OCT monitoring images obtained on day 1, day 5, day 10, and on day 15 respectively. C1 and C2 are the 3D volumetric OCT images of the stipe surface taken on day 1 and on day 15 accordingly. In images from A1-A4 it is quite clearly seen that the cap trama in the day 1 image is less visible as the depth direction increases in the OCT image, but as the days progress and the moisture contents slowly reduces in the mushrooms, the visible optical thickness of cap trama in A3 and A4 images is more. Also, similarly in images B1-B4 the visible stipe trama thickness in the OCT images is gradually increasing as the monitoring days increase. Trama of the mushroom body mostly comprises of water content and essential nutrients for the mushroom growth. As the refrigerating life of the mushroom increases the moisture level in mushroom gradually decreases in time. Thereby, this leads to the shrinking of trama thickness and thus the more layer like structures starts to appear in later day OCT images. Also, due to the refractive index change from day1 to day 15 along with the optical properties of water contained in the mushroom is the reason for the lesser visible structures of the internal mushroom body in OCT images in day 1, which starts to show as increased intensity and visibility of internal structures within the samples. Images C1 and C2 shows the volumetric 3D OCT images of the mushroom on day 1 and day 15 respectively.
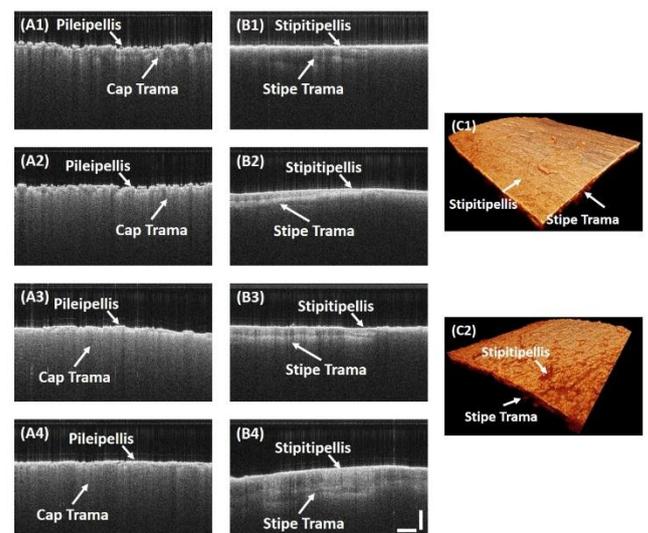

Figure 3: 2D OCT monitoring images of mushroom sample. A1, A2, A3, and A4 and also, B1, B2, B3, and B4 represents OCT images taken on day1, day5, day 10 and day 15 respectively. C1 and C2 are 3D OCT images of stem region taken on day 1 and day 15 respectively. The scale bar is common and represents 500 µm.

To get a better understanding of the internal structural changes of trama layers in mushroom, we did A-scan analysis of the day 1 and day 15 OCT images of mushroom. Images A1 and A2 are the 2D OCT images of pileus region of a mushroom taken on day 1 and day 15 respectively. And A3 is the comparative A-scan plot of the two images A1 and A2. Similarly, B1 and B2 are the 2D OCT images of stipe region of the same mushroom taken on day 1 and day 15 respectively. And B3 is the comparative A-scan plot of the two images B1 and B2. The

regions used for A-scan analysis if highlighted with a red and blue rectangular box in the OCT images. From the generated A-scan plots it can be observed that the visible thickness of cap trama and stipe trama increases on day 15 OCT images and also, the trama forms a dense layer due to the shrinking resultant of moisture loss and the layer formation in trama region increased significantly in day 15 images of the mushroom body. This can be seen in A3 and B3 plot images, where the blue plot represents the A-scan profile of day 1 image and red plot represents the A-scan profile of day 15 images of the mushroom. The increase in visibility of depth in images is due to the shrunken trama thickness due to moisture loss and increasing layer like structure, is also increased on day 15 images. In both the A-scan plots the peaks marked with 1, 2 and 3 are pointed with two arrowheads. The blue arrow heads are peak positions on day 1 image of stipe and cap regions. Similarly, the red arrowheads represent peak positions of day 15 plots. It can be observed that the peak positions of day 15 plots have moved further towards the first peak, indicating the layers getting closer to each other. Also, the intensity of the peaks in lower layers of day 15 is comparatively lesser to day 1 peak intensities, this is due to the dryness state of the sample.
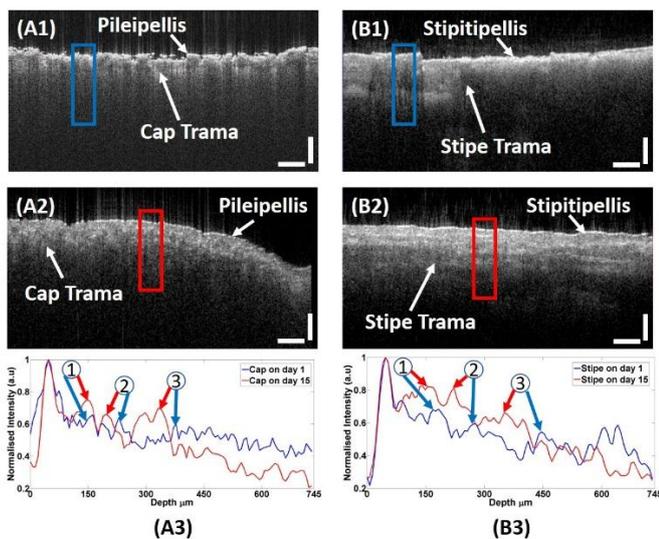

Figure 4: A-scan plot comparison of day 1 and day 15 OCT image of mushroom. A1 and A2 are the 2D OCT images of pileus region taken on day 1 and day 15 respectively, also, B1 and B2 are the 2D OCT images of stipe region of the same mushroom taken on day 1 and day 15 respectively. A3 and B3 are corresponding A-scan plot comparison of pileus region and stipe region. Scale bar represents 500 μm.

CONCLUSION

We monitored *Pleurotus eryngii* mushroom samples (that was refrigerated during the entire experimental duration) for consecutive days using a custom built swept source optical coherence tomography system. Also, the trama thickness level in mushroom body was monitored as the moisture content of the mushroom decreased in time. Mushroom are widely consumed and also, widely used for medicinal purposes. This makes it of high importance to maintain the freshness of the mushroom to the utmost possible level. Through our proposed study we have shown the possible application of using OCT as an optical inspection tool for the moisture level monitoring in mushrooms without any contact or damage to the samples in real-time, and the obtained results was supported with A-scan analysis plots. Also, the proposed study can be used by researches that can be based on the similar applications for different agronomical studies.

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