Monitoring technique for vitamin B₂ and water content in plants for producing vegetables

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Abstract
Optical sensing techniques have become important tools in agricultural engineering to enhance human health and welfare by increasing farm productivity and controlling agronomic practices. If nutrients needed for human health could be monitored in real time, the amount of these nutrients contained in vegetables could be controlled to some extent by adjusting the growth conditions in green houses or open fields.

The authors have developed viable methods to detect the water and vitamin B₂ content in living plants using optical reflection, absorption, or fluorescence monitoring. Compact and mobile equipment has also been developed for this purpose. The equipment consists of light emitting diodes (LEDs), a compact spectrometer, and a fibre optic system with a lens. The optical reflection from, absorption in, or fluorescence from growing leaves were monitored under LED illumination, and the water or vitamin B₂ content could then be measured in growing vegetables.

The vegetables used were mainly Jew’s mallows (Corchorus olitorius) and leaf lettuces, which were grown in our laboratory atmosphere.

The applicability of these techniques to growing vegetables to benefit human health and welfare is demonstrated in this paper, based on practical data.

Keywords: optical sensing, fluorescence, green house

Introduction

Various kinds of optical equipment and systems have been introduced into agricultural fields and used practically to benefit human safety, health and welfare. Light detection and ranging (LIDER), for example, is used to estimate harvest timing or to monitor stones and large holes in crop fields in combination with differential GPS systems. In addition to these applications, optical sensing can possibly be used to increase the harvest of crops and the nutrients in vegetables, and thus contributes to the enhancement of human health and welfare.

Many examples of optical sensing in plants have been reported for monitoring of the sugar content in fruit (G. G. Dull et al., 1989; S. Kawano, et al., 1993; D. C. Slaughter et al., 1996) and other measurement applications (Shyam N. Jha & T.Matsuoka, 2004,; Tian Hai-qing et al., 2007; D.C. Slaughter et al., 2008).

Among these examples, we have already reported vitamin-B₂-content monitoring using fluorescence spectra and water content sensing by monitoring optical reflection in growing plants (M. Fukuda et al., 2008; K. Sasaki et al., 2010). The water supply in green houses and open fields can be controlled precisely if the water content in the plants is monitored. Plant behavior can be studied under various environmental conditions if the water content is precisely monitored. If the content of vitamin B₂ content can be monitored in growing plants, the final content can then be controlled. In this paper, we demonstrate the applicability of
these monitoring techniques for vitamin B$_2$ and water content of plants based on practical data.

Materials and Methods

The Optical characteristics to be monitored, which can be applied to monitor the internal information in growing plants, are optical reflection and scattering, optical absorption, and fluorescence excited under light illumination. These characteristics should be selected while taking the properties of the plant of interest into consideration. If a certain nutrient in a plant has a peculiar absorption wavelength, then the optical absorption monitoring technique can be applied to quantify the content level of that nutrient. If fluorescence emission at a certain wavelength band is generated by the nutrient under light illumination, its intensity is a measure of the nutrient content level in the plants. The change in refractive index within plants can be also calculated by monitoring the change in optical intensity of reflected light. By combining these monitoring techniques, the nutrient content or the growth status of plants can be monitored precisely. Among these optical techniques, we present two techniques, fluorescence intensity monitoring and refractive index monitoring, for plant growth state monitoring applications.

**Fluorescence intensity monitoring**

The optical system used in this study was designed to be compact and mobile to enable easy monitoring of vitamins in all parts of vegetables (see Fig. 1). The optical source is a light-emitting-diode (LED) emitting 365-nm-band UV light, and the fluorescence emitted by vitamin B$_2$ and the other components contained in the leaf was coupled into a silica optical fiber and guided into a spectroscope (USB2000, Ocean Optics) to monitor the spectrum. The spectroscope was compact (weighing less than 200 g) and was controlled with a personal computer. The light passing through the leaf consisted of fluorescence and the excitation light. A UV-cut filter (ITY430, ISUZU GLASS) was therefore inserted between the sample and the silica optical fiber. We selected Jew’s mallow (*Corchorus olitorius*) as a plant sample because of its relatively high vitamin B$_2$ content (0.42 mg per 100 g of edible portions of the plant).

![Figure 1 Fluorescence measurement system. The UV-light emitted from the LED is focused on a leaf to be monitored, and the fluorescence spectrum is measured with a computer-controlled spectrometer.](image-url)
Refractive index monitoring

The refractive index change in plants is monitored by using optical fiber system shown in Fig. 2. A laser diode lasing at 650 nm was used to eliminate optical absorption of the water and sugar contained in plants. The output light from the laser was guided into a fiber with a lens and then into an optical fiber probe. The fiber probe was made of conventional 125 μm diameter multimode silica fiber, and the fiber tip was cleaved into a flat mirror. The guided light was partially reflected at the fiber tip in accordance with the reflectivity determined by the refractive index difference between the fiber and the material of the plant into which the fiber was inserted, as shown in Figs. 2 and 3.

![Figure 2](image.png)

**Figure 2** Measurement system for refractive index of inner parts of plants. The fiber probe tip is inserted into a plant, and the amount of back-reflected light is monitored with a photodiode. The amount of back reflection depends on the reflectivity R determined by the refractive index of the fiber and the plant material.

**Figure 3** Optical fiber probe inserted into a lettuce leaf. The lasing light at 630 nm is observed at the tip of the probe in the leaf.
The reflected light, which is determined by the reflectivity, $R$, was guided again through the optical fiber was detected using a photodiode. The optical signal is converted into an electrical signal, which was amplified using a lock-in amplifier and then recorded. The vegetable samples used were commercially available leaf lettuces. These lettuces were set in an apparatus composed of a chamber, a CO$_2$ monitor, and a thermocouple. The fiber tip of the monitoring system was inserted into a leaf, and the inserted part was immediately closed and sealed with an organic material to prevent water evaporation.

Results

**Fluorescence intensity monitoring**

Figure 4 shows the fluorescence spectrum from the Jew’s mallow sample measured using the experimental system shown in Fig. 1. The vertical axis is normalized with respect to the maximum fluorescence intensity at a wavelength of 687 nm. The light at wavelengths lower than 400 nm was filtered out with the UV-cut filter shown in Fig. 2.

![Fluorescence spectrum from Jew’s mallow.](image)

In Fig. 4, three fluorescence peaks can be observed, a broad peak centered at a wavelength of 530 nm, a sharp peak at 690 nm, and a broad peak between 700 and 740 nm. Two fluorescence peaks in 690 nm and between 700 and 740 nm originate from chlorophyll. The fluorescence peak at 530 nm was observed in the fluorescence spectrum of living leaves gathered from various part of a Jew’s mallow plant. The peak wavelength at 530 nm was estimated to be the fluorescence of vitamin B$_2$.

However, Jew’s mallow contains other fluorescence compounds that have the same fluorescence band as vitamin B$_2$, such as β-carotene. The experimental system shown in Fig. 2 could also detect β-carotene’s fluorescence. The β-carotene content is approximately 25 times higher than that of vitamin B$_2$ in Jew’s mallow. However, even with this content ratio, the fluorescence intensity of vitamin B2 was approximately 20 times larger than that of β-carotene. Therefore, the β-carotene fluorescence intensity contribution could be ignored in the fluorescence spectrum. The relationship between the peak fluorescence intensity in the spectra and the contents of the Jew's mallow was also confirmed by using high-performance liquid chromatography.

This technique was extended to the monitoring of vitamin B$_2$ in leaves at various growing stages. Figure 5 shows the fluorescence spectra of young, green, and senescent Jew’s
mallow leaves under ultraviolet illumination at 365 nm, and that of a vitamin B\textsubscript{2} solution as a reference. To compare the vitamin B\textsubscript{2} spectra of the leaves, the fluorescence intensities were normalized at 620 nm because this wavelength was not affected by the fluorescence of vitamin B\textsubscript{2} (at 530 nm) and chlorophyll (at 680 and 740 nm). A peak was observed at 530 nm in the fluorescence spectra of both the young and the green leaves, and the fluorescence intensity was lower for the young leaves than for the green leaves. There was no peak at 530 nm in the spectrum of the senescent leaves. These results show that young leaves contain less vitamin B\textsubscript{2} than green leaves, and that senescent leaves do not contain a detectable level of vitamin B\textsubscript{2}. These results clearly show that the optical system developed in this study can be applied to monitor vitamin B\textsubscript{2} in living plants and can contribute to human health and welfare by control of the growth conditions and harvest time of crops and by increasing their nutrient content.

Figure 5: Fluorescence spectra of young, green, and senescent Jew’s mallow leaves, and of vitamin B\textsubscript{2} solution as reference.

Refractive index monitoring

A typical change in refractive index in a leaf lettuce is shown in Fig. 6 as a function of elapsed time. Here, the refractive index is translated into water content using the Clausius-Mossotti relation under the assumption that only water content changes (Fukuda M. et al., 2008). The leaf lettuce was set in a dark chamber at room temperature. Before monitoring, the lettuce remained in the chamber for 48 hours without a water supply or light illumination. The water content in percentage units was calculated from the refractive index change, which was calculated from the change in magnitude of optical feedback. At this point, we hypothesized that the refractive index in plants is proportional to the concentration of liquid in the plants and the minimum value of the refractive index is 1.332 corresponding to pure water. This hypothesis can be used with any normal solution, such as a sugar solution.

The water content in the leaf lettuce was nearly constant after monitoring started, and it quickly increased after a certain time period (about 45 min after supplying water). The increase in the water content became saturated after the quick increase. The time interval between the water supply and the change in the water content corresponded to the time taken for the water supplied from the roots to reach the monitoring point in the leaf. When the water reached the monitoring point, the reflectivity at the fiber tip increased because the refractive index in the lettuce leaf decreased. This behavior corresponds to the change in the refractive index.
index and the water content in the leaf. These results demonstrate that the optical back-reflection monitoring method is sensitive to refractive index change and thus to the water content of the leaves.

![Graph showing change in water content in leaf lettuce.](image)

**Figure 6** Change in water content in leaf lettuce. The refractive index monitored and is translated to water content.

**Conclusions**

We have developed techniques to monitor nutrient levels and water content of growing vegetables and have confirmed their applicability to living crops. The systems using our technique will help to produce nutrient-rich vegetables in green houses and/or fields and enhance human health and welfare. This system will be also useful for determination of optimal harvest times by monitoring the nutrient contents of growing vegetables and will therefore assist the work of farmers.

**References**


