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In Situ Estimation of the Constituents of Green Soybean (Edamame) Pod using Near-Infrared Transmission Spectroscopy

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Abstract

Purpose: We estimated the dietary qualities of green soybean (edamame) by using a specialized NIR transmission spectrometer to determine the constitutive properties of the soybean, such as the sucrose content and ninhydrine reaction quantity (NRQ; defined by the ninhydrine reaction, which has a high positive correlation with the total free amino acids), with the purpose of establishing a quality assurance system. **Methods:** We used a newly developed spectrometer probe that enables in situ estimation of the constituents of the soybean. **Results:** The calibration results obtained using a wavelength range of 760-960 nm were characterized by R^2 = 0.57 and standard error of cross-validation (SECV) of 0.78% for sucrose, and R^2 = 0.59 and SECV = 0.35% for NRQ. **Conclusions:** These results are inferior to those of our previous study obtained using a specialized bench-type transmission spectrometer. The poorer results are attributed to several possible reasons, including the effect of direct sunlight and the unstable sample presentation. We plan to conduct further study using improved optical layout and sample presentation.

Keywords: Edamame, Green soybean, In situ constituent estimation, Near-infrared transmission spectroscopy, PLS regression modeling

Introduction

The production and consumption of green soybean (edamame) is on the increase in Japan owing to the present national consciousness of healthy eating. Our laboratory is located in an area that produces a famous local variety of green soybean known as “dadachamame,” which is highly praised by connoisseurs. The reputation of dadachamame stems from its pleasant flavor and rich dietary qualities such as the sucrose content and ninhydrine reaction quantity (NRQ; defined by the ninhydrine reaction, which has a high positive correlation with the total free amino acids). However, edamame is a vegetable with a high respiration rate, which causes the dietary qualities to drastically depreciate within a few days of harvest (Iwata and Shirahata, 1979). Hence, with the increasing production of dadachamame, there is the need to improve its quality assurance system.

Several studies have been conducted to investigate the near-infrared (NIR) spectroscopy used to estimate the quality of beans and seeds. NIR spectroscopy has many advantages such as being nondestructive, simple, and fast, and is therefore considered adequate for quality assurance systems. Velasco et al. found that the fatty acid composition of the oil in intact-seed mustard could be estimated with a high degree of accuracy using NIR spectroscopy (Velasco et al., 1997). Perez-Vich et al. (1998) carried out a methodological study on the seed oil content and fatty acid composition of sunflower, and found that the accuracy when intact seeds were used was less than that for meal, but was sufficiently reliable for prescreening. Sato et al. examined the feasibility of NIR spectroscopy for estimating sesame seed fatty acid (FA) composition, and reported that it was reasonably accurate for breeding purposes (Sato et al., 2003). Golebiowski (2004) and Golebiowski et al. (2005)

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used NIR spectroscopy to investigate the oil in intact canola seed and discussed the attributes of the spectrum and the relationship between the principal components and the oil content. Arganosa et al. (2006) found that NIR reflectance spectroscopy was sufficiently accurate for determining the crude protein content of field peas for a breeding program. Font et al. (2006) also investigated the potential of NIR spectroscopy for determining the acid detergent fiber (ADF), fatty acid, and oil contents of oilseed, and found that the accuracy was suitable for screening. However, no study has been conducted to examine the dietary qualities of edamame, which has a very high moisture content (>60%), the exception being our research to estimate the overall flavor of green soybean by single pod measurement (Natsuga et al., 2007).

Since 2002, we have evaluated the dietary qualities of green edamame using a commercial near-infrared transmission (NIRT) analyzer, and have succeeded in obtaining good estimates of both the sucrose content and NRQ (Sue et al., 2009; Egashira et al., 2011). However, it was necessary to develop an NIR spectrometer for determining the contents of a single-pod green soybean in order to shorten the breeding time and enhance decision making regarding the optimal harvest time. Until we embarked on our investigation, no such instrument existed. We began the development of an NIRT spectrometer specifically for this purpose in 2004. The developed NIR transmission spectrometer is reasonably accurate for estimating both sucrose content and NRQ (Natsuga et al, 2007). We also used the developed NIRT spectrometer to demonstrate the quality changes that occur during storage (Maebashi et al., 2012).

Unfortunately, the instrument is bench-type and the spectral measurement can only be done in the laboratory. Hence, in the present study, we developed a new probe that enabled in situ estimation of the constituents of green soybean.

Materials and Methods

Spectrometer

A spectrometer consisting of a monochromator, light source, and fiber optic probe was used for spectral measurement of green soybean standing in the field. The view of the spectrometer and details of the probe are shown in Figures 1 and 2, respectively. The specifications of the spectrometer are given in Table 1. The spectrometer was carried in a basket and brought into the field. The light from the light source is guided by an optical fiber bundle and used to illuminate a pod. The light passes through the

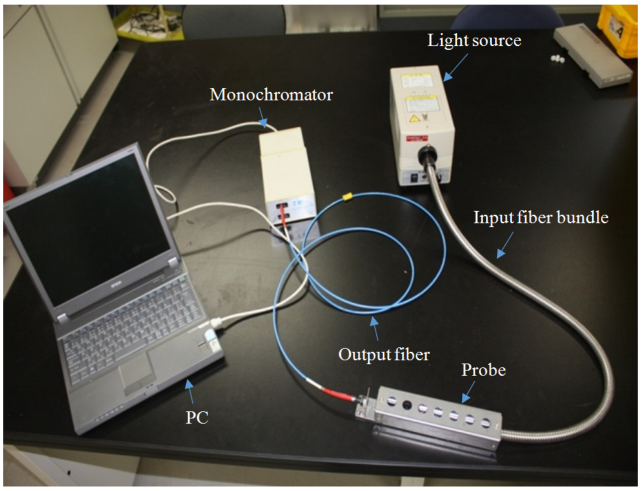

Figure 1. View of the spectrometer.

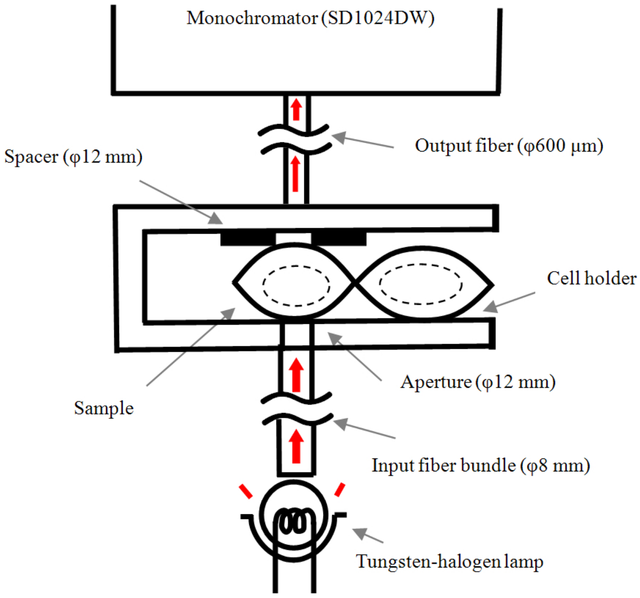

Figure 2. Details of the probe.

Table 1. Specifications of the spectrometer

PC	NA101 (EPSON, Japan)
Output fiber	P600-2-VIS/NIR (Ocean Optics Inc., USA)
Input fiber bundle	LI-SE-LGA1-8 (Fortissimo Co., Japan)
Light source	LA150-UE (Hayashi Clock Works, Japan)
Spectroscopy	OOI SD1024DW (Ocean Optics Inc., USA)
Data acquisition software	OOI Bace32 (Ocean Optics Inc., USA)

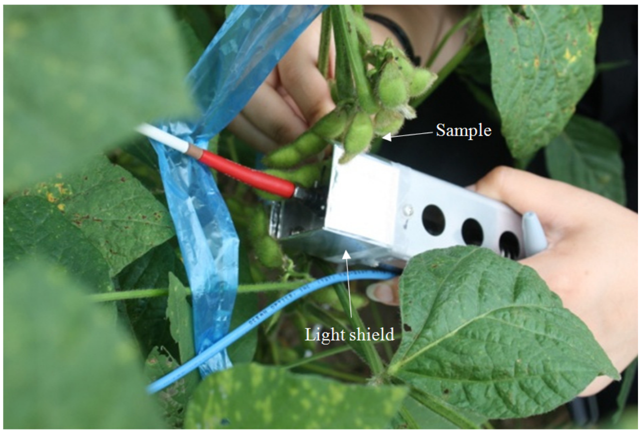

Figure 3. Spectral measurement in the field.

pod and kernel, and is then delivered into the spectrometer via another optical fiber. The wavelength range applied in the present study was 650-1100 nm, and the number of pixels was 1024. Figure 3 shows the in situ spectral measurement.

Samples and method

Samples

Five types of domestically grown cultivars/varieties comprising four dadachamame cultivars and a commercially available soybean cultivar were harvested at the Takasaka experimental farm of the Faculty of Agriculture, Yamagata University in 2013 and used for the study. A total of 56 samples were used to calibrate the model of the developed spectrometer.

Spectral measurement

The spectra were collected over three consecutive days with the purpose of estimating the optimal harvest day. A 10-mm-thick Teflon® plate was used as the spectral reference. A sample was inserted into the probe and its position was fixed using a spacer inserted between the sample and the output optical fiber. A light shield was fitted around the sample. The time of a single exposure was 3 ms, and the exposure was repeated 100 times. The spectra were then converted into data with regular intervals of 1 nm using a specially developed computer software.

Reference analysis

The samples were freeze-dried and ground using a vibrating sample mill (TI-100, Heiko, Japan). The ethanol extraction was then carried out. The sucrose content was subsequently measured using a Sucrose/D-Glucose Test Kit (Roche, Switzerland), and the NRQ was determined by the ninhydrine reaction method. The procedures were as in previous studies (Natsuga et al., 2007; Sue et al., 2009; Egashira et al., 2011; Maebashi et al., 2012).

Calibration

The calibrations for both sucrose content and NRQ were done by partial least squares (PLS) regression modeling using the full cross-validation method and The Unscrambler 9.2 software (CAMO, Norway). We examined various wavelength ranges in the obtained spectra and the best calibrations were selected.

Results and Discussion

Figure 4 shows the original spectra and their second derivative for a wavelength range of 750-1100 nm obtained by the spectrometer. Wavelengths shorter than 750 nm were excluded from the calibration because smooth spectra could not be obtained in that range. A peak was observed in the assigned water absorption around 960 nm. Parts of the calibrations for sucrose content and NRQ are given in Tables 2 and 3, respectively. The calibration results for a

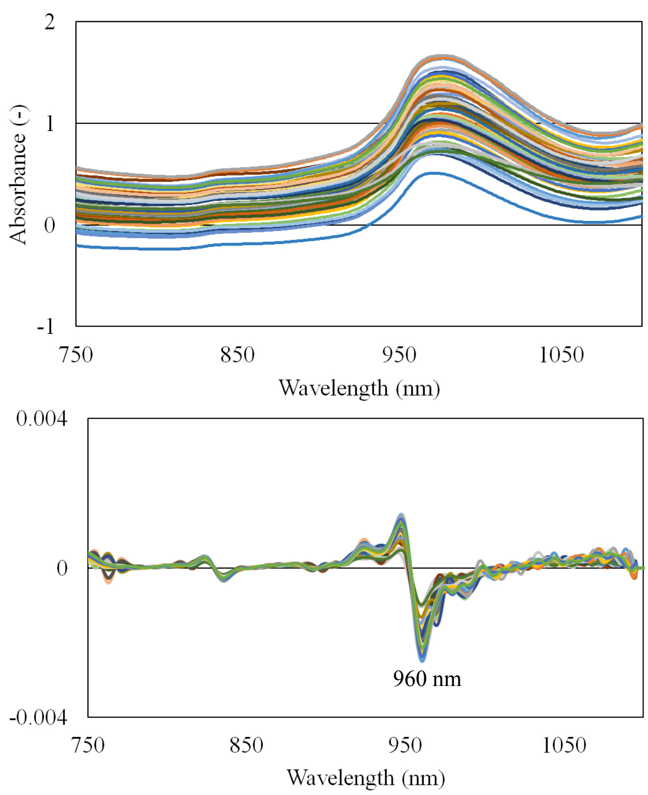

Figure 4. Original and second derivative of the spectra obtained by the spectrometer (750-1100 nm).

wavelength range of 760-960 nm were characterized by R^2 = 0.57 and standard error of cross-validation (SECV) of 0.78% for sucrose, and R^2 = 0.59 and SECV = 0.35% for NRQ. Figures 5 and 6 show the respective scatter plots. As can be observed from Tables 2 and 3, both the sucrose content and NRQ calibrations produced better estimation accuracies for a wavelength range of 750-1100 nm. They were, however, not adopted because the calibration coefficients for wavelengths above 1100 nm were very noisy and unstable (not shown here). The present results are inferior to those of our previous study, which were characterized R^2 = 0.74 and SECV = 0.39% for sucrose content, and R^2 = 0.69 and SECV = 0.21% for NRQ, obtained using the special bench-type transmission spectrometer in the laboratory (Natsuga et al., 2007). The poorer results of the present study are attributed to several possible reasons, including the effect of direct sunlight and the unstable sample presentation. Nevertheless, this being the first attempt at using the spectrometer in the field for in situ estimation of green soybean quality, we are optimistic that improvements can be achieved.

Table 2. Calibration results for sucrose content

Wavelength range	Number of samples	nF*	R^2**	SECV*** (%)
750-1100		7	0.60	0.74
750-1050		7	0.54	0.80
750-1000	56	6	0.53	0.80
750-960		6	0.57	0.78
750-950		12	0.61	0.74

* Number of factors
** Coefficient of determination
*** Standard error of cross-validation

Table 3. Calibration results for NRQ

Wavelength range	Number of samples	nF	R^2	SECV (%)
750-1100		13	0.64	0.33
750-1050		6	0.55	0.36
750-1000	56	5	0.56	0.36
750-960		4	0.59	0.35
750-950		4	0.58	0.35

Conclusions

We developed a new optical probe for the in situ estimation of the constituents of green soybean. The calibration results for a wavelength range of 760-960 nm were characterized by R^2 = 0.57 and SECV = 0.78% for sucrose content, and R^2 = 0.59 and SECV = 0.35% for NRQ, respectively. These results were not very encouraging owing to several factors that possibly had significant effects on the spectral measurement, such as direct sunlight and unstable sample presentation. Nevertheless, this being the first attempt to use the spectrometer in the field for in situ estimation of the quality of green soybean, we are optimistic that impro-

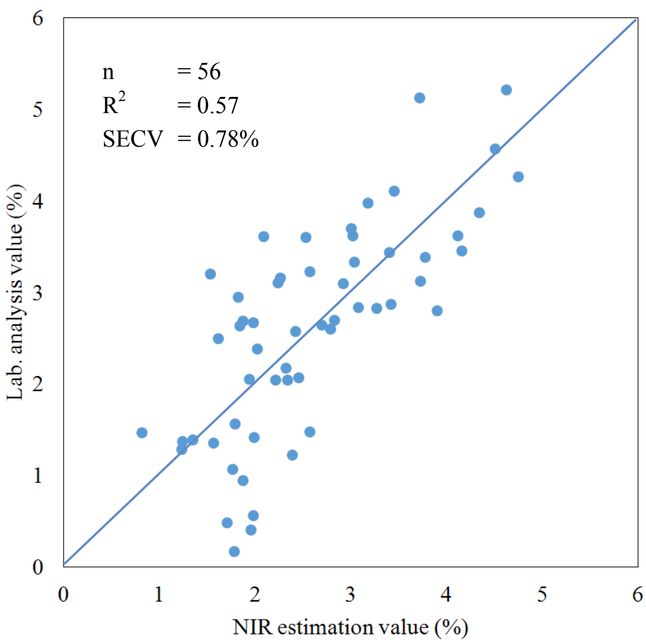

Figure 5. Scatter plot for sucrose content (750-960 nm).

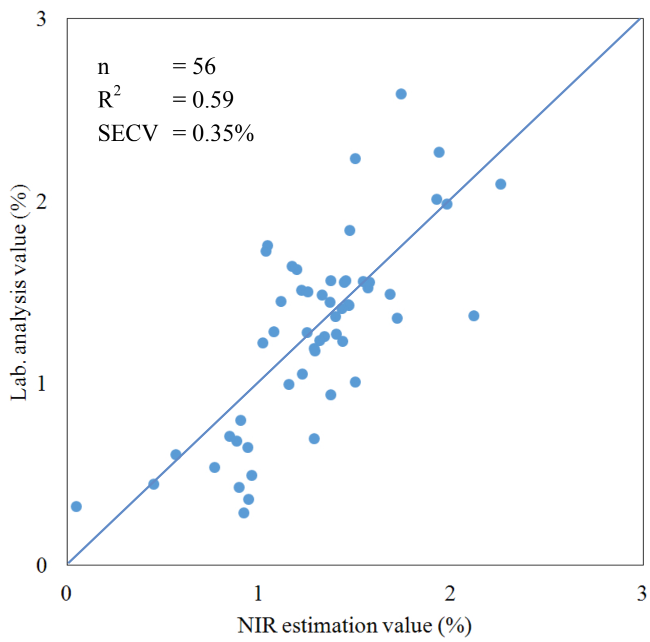

Figure 6. Scatter plot for NRQ (750-960 nm).

vements can be achieved. Further study is planned to improve the optical layout and sample presentation.

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