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Estimation of the Flavor of Green Soybean during Storage from Single Pod Measurements using Dedicated Near-Infrared Transmission Spectrometer

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Abstract

Purpose: Green soybeans (edamame) are now an economically important and popular food product in Japan. In order to shorten breeding time and to decide an optimal harvest time, we have been developing a dedicated NIRT spectrometer since 2004 for the determination of constituent content such as sucrose and free amino acids, which are two major contributors to the eating quality, in a single pod green soybean. **Methods:** The obtained models showed that the developed NIRT instrument had reasonable accuracy for the determination of these two components. Then we carried out the investigation into the change in two components during a few days storage using these models with changing time, variety/cultivar, packaging and temperature. **Results:** The result showed that the most affecting factor on decreasing both sucrose content and free amino acids was variety/cultivar. The time, packaging and temperature also affected significantly in most cases.

Keywords: Near-infrared transmission (NIRT) spectroscopy, Green soybean, Single pod, PLS regression, Constituent content change, Storage

Introduction

The production and consumption of green soybeans (edamame) has been increasing in Japan due to continuing national health-conscious food trends. In addition, our laboratory is located in an area producing a famous local brand of green soybeans, named “dadachamame”, that is very popular and highly praised by connoisseurs. Its reputation comes from its pleasant flavor and richness of eating quality-related constituents such as sucrose and ninhydrine reaction quantity (NRQ), which has a high positive correlation with total free amino acids. However, edamame is a vegetable with a high respiration rate, and thus its eating quality-related constituents such as sucrose and NRQ tend to decrease drastically within a few days of harvesting (Iwata and Shirahata, 1979). Therefore, as the production of “dadachamame” increases, quality assurance systems must be improved.

Several researchers have been investigating near-infrared (NIR) spectroscopy for estimating the quality of beans and seeds. NIR spectroscopy has many advantages such as being nondestructive, simple, and quick, and is therefore considered adequate for quality assurance systems. Velasco *et al*. (1997) found that fatty acid composition of the oil in intact-seed mustard could be estimated with a high degree of accuracy using NIR spectroscopy. 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 using intact seeds was less than that of meal, but was reliable enough for the purpose of prescreening. Sato *et al*. (2003) examined the feasibility of NIR spectroscopy for the determination of sesame seed fatty acid (FA) composition and reported that it could be estimated with reasonable accuracy for breeding. Golebiowski *et al*. (2004, 2005) investigated oil in intact canola seed using NIR spectroscopy and discussed the attributes of the

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spectrum and the association between principal components and oil content. Arganosa *et al*. (2006) found that the accuracy of NIR reflectance spectroscopy for the determination of crude protein content of field peas was adequate for a breeding program. Font *et al*. (2006) investigated the potential of NIR spectroscopy for determining acid detergent fiber (ADF), fatty acid composition, and oil content of oilseed and found that the accuracy obtained was suitable for screening. However, no studies have examined the eating quality-related constituents of edamame, which has a very high moisture content (>60%), except for our research to estimate the overall flavor of green soybean from a single pod measurement. (Natsuga et al., 2007)

We have been evaluating the eating quality of green edamame using commercial near-infrared transmission (NIRT) analyzer since 2002 and succeeded to obtain good estimations for both sucrose and free amino acid content which are two major contributors to the eating quality. (Sue et al., 2009; Egashira et al., 2011) However, a NIR spectrometer for the determination of constituent content of a single pod green soybean is required in order to shorten breeding time and better decide the optimal moment for harvesting, but until we started to investigate no such instruments were available. Consequently, we have been working on the development of a NIRT spectrometer dedicated to this purpose since 2005. Developed NIR transmission spectrometer had reasonable accuracy for both sucrose and NRQ (defined by ninhydrine reaction, which has a high positive correlation with total free amino acids) (Natsuga et al., 2007). In this paper we introduce the result from the investigation of the quality changes during storage using the developed NIR transmission spectrometer.

Materials and Methods

Spectrometer

The optical layout of the developed NIR transmission spectrometer was shown in Figure 1 as described SOMA instrument in the previous study (Natsuga et al., 2007). The instrument was manufactured by Soma Optics, Co., Ltd (Tokyo, Japan). A tungsten-halogen lamp is placed right at the foot of sample stage. A light beam illuminates a pod and passes through the pod and kernel, and is then collected into the spectrometer via optical fiber. A wavelength range was 710-1045 nm with 1024 pixels.

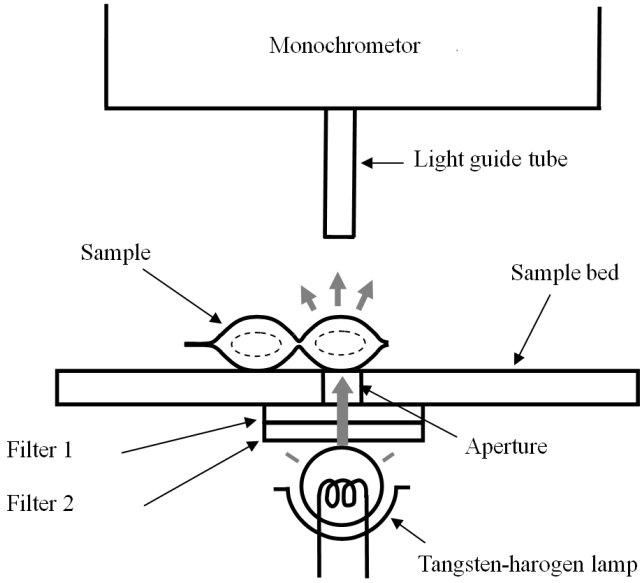

Figure 1. Optical layout of the developed NIR transmission spectrometer.

The exposure time was 10 ms. A single exposure was repeated for 500 times. The spectra were then converted into 1 nm regular interval data with specially developed computer software.

Materials and Methods

Calibration development

A total number of samples used for calibration model development were 752 for sucrose and 758 for NRQ, respectively. The numbers of samples between constituents were not consistent with each other since some samples were excluded from each calibration model as outliers. They consisted of 56 domestically grown cultivars/varieties, among them 25 were dadachamame cultivars and 31 were commercially available soybean cultivars, harvested in the Takasaka experimental farm of Faculty of Agriculture, Yamagata University between 2005 and 2009. They include room temperature samples, temperature fluctuation samples and storage samples.

Quality change during storage

A total of six varieties/cultivars of domestically grown soybeans were used. They consisted of four dadachamame cultivars collected around Tsuruoka and commercially available two soybean cultivars. Four pods, with two beans each, were chosen from the same plant for the repetition. The sepal side bean was used for sucrose content analysis and the other was used for NRQ analysis,

Figure 2. MA packaged green soybean sample.

respectively. Samples were placed in the room (18-28°C) and in the refrigerator (4°C) during three days (72 hours) storage with MA (Modified Atmosphere) packaging using P-Plus (Sumitomo Bakelite Co., Ltd., Tokyo, Japan) and without it (i.e. open-air.) Samples were sealed using Polysealer P-200 (Fuji Impulse Co., Ltd., Osaka, Japan.) Spectra were obtained every six hours during storage. Figure 2 shows the sample in MA packaging.

Reference analysis

In the calibration development, two randomly chosen sets of samples (approximately 10 g) were analyzed. In the experiment of the quality change during storage, each kernel was analyzed. Samples were freeze-dried and were ground using vibrating sample mill (TI-100, Heiko, Japan). The ethanol extraction was then carried out. The sucrose content was determined by Sucrose/D-Glucose Test Kit (Roche, Switzerland) and the NRQ was determined by ninhydrine reaction method. These were the same as the previous study (Sue et al., 2009; Egashira et al., 2011; Natsuga et al., 2007).

Calibrations and Estimations

Calibrations for both sucrose content and NRQ were developed by partial least squares (PLS) regression modeling with full cross validation methods using The Unscrambler 9.2 (CAMO, Norway) software. We checked various wavelength ranges in the obtained spectra and the best calibrations were selected. Developed calibrations were then used for the estimation of quality change during storage.

Results and Discussion

Developed calibrations

Figure 3 and Figure 4 show the calibration for sucrose

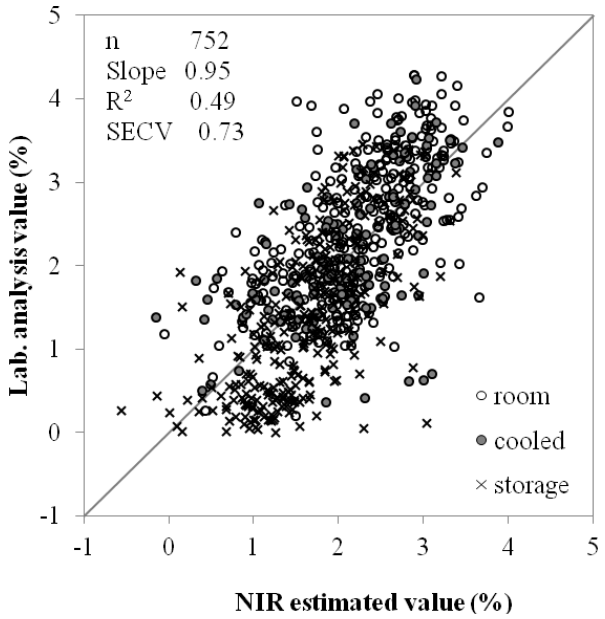

Figure 3. Sucrose content calibration (850-1040 nm)

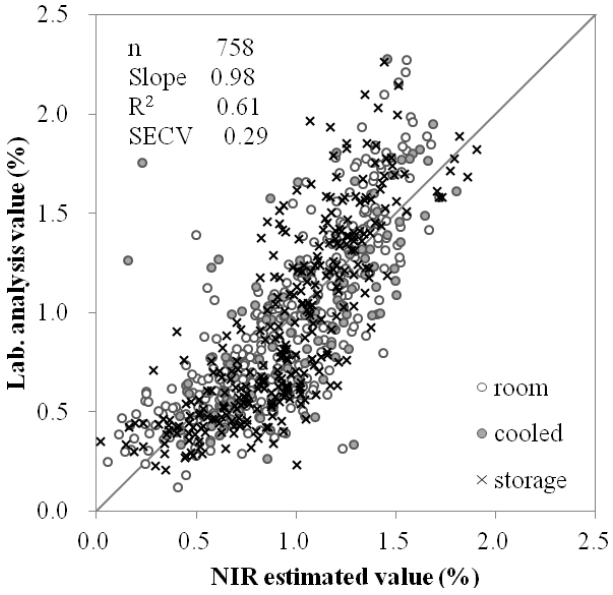

Figure 4. NRQ calibration (750-1040 nm)

content and NRQ with their wavelength range, respectively. There were no particular tendencies such as bias, skew and stratification regarding both temperature and time; hence they were considered as applicable to the estimations during storage and were used for the estimation of sucrose content and NRQ. The estimation results were then statistically analyzed using SAS (SAS Institute Inc., Cary, USA). In the four-way ANOVA with factors as time, variety/cultivar, packaging and temperature, only the interactions between two factors were considered

since higher interactions were very complicated and were very difficult to interpret.

Quality change during storage

Sucrose content

A four-way factorial ANOVA results for sucrose content in 2008 and 2009 were summarized in Table 1 and 2, respectively. The most contributing factor was variety/cultivar in both years. Other three factors, time, packaging and temperature also significantly contributed except for packaging in 2008. As for interactions, the results were different between two years. Only time x packaging was not significant in 2008 while time x packaging, variety/cultivar x temperature and packaging x temperature were not significant in 2009. They might reflect the difference of growing condition in two years. Figure 4 shows the sucrose content changes during storage for the cultivar Shonai 5 in 2009. The sucrose content showed significant reduction after three days (72 hours) room temperature storage while significant reduction was not observed in the sample stored in the refrigerator. Masuda (2003) reported that the sucrose content of green soybean stored at 27℃ without packaging decreased from approximately 3% to 1% in two day storage after harvesting. Our result is consistent with his result.

NRQ

A four-way factorial ANOVA results for NRQ content in 2008 and 2009 were summarized in Table 3 and 4, respectively. The most contributing factor was variety/

Table 1. ANOVA result for sucrose content in 2008

Source[1]	DF[2]	Sum of squares	Mean square	F value[3]	Contribution (%)
A	12	12.744	1.062	6.4 ***	1.8
B	5	272.625	54.525	326.1 ***	46.4
C	1	0.004	0.004	0.0 ns	0.0
D	1	44.150	44.150	264.0 ***	7.5
A x B	60	23.292	0.388	2.3 ***	2.3
A x C	12	3.341	0.278	1.7 ns	0.2
A x D	12	12.522	1.044	6.2 ***	1.8
B x C	5	17.189	3.438	20.6 ***	2.8
B x D	5	9.552	1.910	11.4 ***	1.5
C x D	1	0.749	0.749	4.5 *	0.1
Error	1133	163.394	0.144		31.3

[1] A: Time, B: Variety/Cultivar, C: Packaging, D: Temperature
[2] Degree of Freedom
[3] *, **, ***, ns mean that it is statistically significant at 5%, 1%, 0.01% and not significant, respectively

Table 2. ANOVA result for sucrose content in 2009

Source[1]	DF[2]	Sum of squares	Mean square	F value[3]	Contribution (%)
A	12	13.778	1.148	7.3 ***	2.9
B	4	158.747	39.687	250.8 ***	39.1
C	1	6.675	6.675	42.2 ***	1.6
D	1	28.452	28.452	179.8 ***	7.0
A x B	48	24.636	0.513	3.2 ***	4.2
A x C	12	2.915	0.243	1.5 ns	0.3
A x D	12	4.744	0.395	2.5 *	0.7
B x C	4	14.579	3.645	23.0 ***	3.4
B x D	4	0.833	0.208	1.3 ns	0.0
C x D	1	0.546	0.546	3.5 ns	0.1
Error	939	148.603	0.158		40.6

Table 3. ANOVA result for NRQ in 2008

Source[1]	DF[2]	Sum of squares	Mean square	F value[3]	Contribution (%)
A	12	1.123	0.094	2.2 **	0.3
B	5	137.258	27.452	647.3 ***	67.2
C	1	0.590	0.590	13.9 ***	0.3
D	1	8.026	8.026	189.3 ***	3.9
A x B	60	2.433	0.041	1.0 ns	-0.1
A x C	12	0.210	0.017	0.4 ns	-0.1
A x D	12	1.878	0.156	3.7 ***	0.7
B x C	5	3.953	0.791	18.6 ***	1.8
B x D	5	0.393	0.079	1.9 ns	0.1
C x D	1	0.087	0.087	2.1 ns	0.0
Error	1133	48.048	0.042		25.9

Table 4. ANOVA result for NRQ in 2009

Source[1]	DF[2]	Sum of squares	Mean square	F value[3]	Contribution (%)
A	12	3.330	0.277	7.6 ***	2.9
B	4	45.064	11.266	307.0 ***	44.8
C	1	0.013	0.013	0.4 ns	0.0
D	1	6.888	6.888	187.7 ***	6.8
A x B	48	2.489	0.052	1.4 *	0.7
A x C	12	0.497	0.041	1.1 ns	0.1
A x D	12	0.548	0.046	1.2 ns	0.1
B x C	4	3.236	0.809	22.0 ***	3.1
B x D	4	1.780	0.445	12.1 ***	1.6
C x D	1	1.874	1.874	51.1 ***	1.8
Error	940	34.497	0.037		38.0

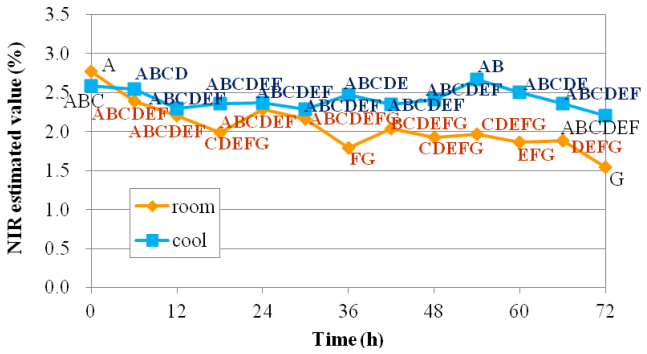

Figure 5. Sucrose content changes during storage (Cultivar: Shonai 5) in 2009.

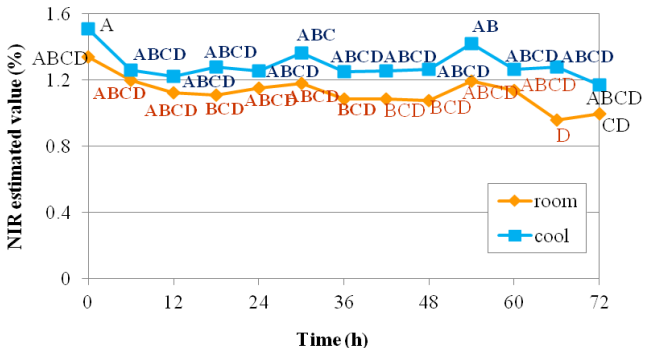

Figure 6. NRQ changes during storage (Cultivar: Shonai 5) in 2009.

cultivar in both years as the same as sucrose content. Other two factors, time and temperature also significantly contributed however packaging did not contribute significantly. As for interactions, the results were different between two years. Time x temperature and variety/cultivar x packaging were significant in 2008 while time x variety/cultivar, time x temperature were not significant in 2009. They might reflect the difference of growing condition in two years. Figure 5 shows the NRQ changes during storage for the cultivar Shonai 5 in 2009. The NRQ

showed no significant reduction after three days (72 hours) in both room temperature storage and refrigerator. Masuda (2003) reported that amino acid such as glutamic acid and alanine fluctuated during two days storage in room temperature and that there were no reductions in these amino acids. He also concluded that the even though amino acid content did not show significant reduction, the sucrose content reduction may contribute to the deterioration of the eating quality since there was definite deterioration of the eating quality after two days storage. Our result was consistent with Masuda's result.

Conclusion

The developed NIR transmission spectrometer could be applicable for the estimations of eating quality-related constituents such as sucrose content and NRQ during storage with/without MA packaging and with/without cooling. ANOVA results showed that the most contributing factor for the quality changes during three days storage was variety/cultivar. Other three factors, time, packaging and temperature also had significant effect for both constituents in most cases. Unfortunately, developed NIR transmission spectrometer is bench-type and can only be used in the laboratory. Our next aim then will be a development of a battery operated portable NIR transmission spectrometer which can be used in the field.

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