Determination of the viability of a grain of soybean using near infrared spectroscopy

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Introduction

Soybean is one of the important grains as food and feed because soybean is nutritionally rich and contains about 40% protein and about 20% lipid. The degree of self-sufficiency in soybean is only 3% in Japan. About 4.9 million tons of soybeans are imported from USA, Brazil, China and other countries. Quality losses of soybean during post-harvest handling were caused by microbial infection, insect attacks and biological aging. The quality of soybean is also affected by the temperature and the humidity during transportation and storage.[1] Measurement of the quality of soybean with accuracy is very important for soybean processing factory such as tofu, soybean paste (miso), fermented soybeans (natto) etc. Quality evaluation of soybean is carried out on the points of physical appearance that contains seed size, seed shape, seed coat, and seed and hilum colour. In an automatic classifier, soybean is selected using these indicators. The second indicator of quality evaluation is the composition of soybean that contains oil content, protein content, fatty acids composition and types of storage proteins. These indicators are not suitable for routine work because the procedures of the analysis of these indicators are complex and have high time-consumption and high running cost. The third indicator is biological activity. Germinability and germination percentage are measured to evaluate the quality. Germinated soybean has a high quality and vice versa. Germination test usually requires seven days. Because the test is too long to evaluate the soybean quality in the soybean processing plant, it is desired to shorten the test term.

Near infrared spectroscopy (NIR) is widely used for rapid and non-destructive analysis in industries, such as agriculture, food, pharmaceuticals, textiles, cosmetics, petroleum and polymer production.[2] Moisture, fat and protein contents of soybean have been measured using NIR.[3]

In this study, a near infrared spectrum of a single grain of soybean was measured and then the germinability of the soybean was tested using the germination test. Discriminant analysis was carried out on the near infrared spectra data and the results of the germination test to get the information of the viability of a single grain of soybean.

Materials and methods

Soybean

Glycine max (L.) ver. Vinton81 used in this study was cultivated in USA in 2001. To accelerate the aging of soybean, the soybean was stored at 40°C and relative humidity of 70%.

Near infrared spectroscopy

A single grain of soybean was put in a single kernel cup (Bran+Luebbe Co., Germany). The absorbance from 4008 cm^{-1} (2495 nm) to 9996 cm^{-1} (1000 nm) was measured at 12 cm^{-1} interval at

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room temperature with a near infrared spectrophotometer equipped with an optical fibber probe (InfraProver II, Bran+Luebbe Co.). The spectrum of each sample was measured 20 scans and averaged. To correct the shift of the baselines of the spectra, second derivative spectra ($d^2\log(1/R)$) were obtained numerically from the raw spectra. Here, R is the reflectance value of the grain at each wavelength.

Germination test

After measuring the spectrum of soybean, the viability of the grain of soybean was examined using the germination test with a roll-paper method.[4] Maximum 25 seeds (five seeds on vertical line × five seeds on horizontal line) of soybean were put on two sheets of wet kitchen paper towel (227 × 224 mm, WR222, Nepia Co., Tokyo) and rolled in. The rolled paper was hung on a stainless steel net put in a beaker with a clothespin. The beaker was covered with a vinyl bag to prevent the rolled paper from drying and was stored at 25°C. Water was sometimes added on the rolled paper to get it wet during the test term, for seven days.

Germinated seed was counted after opening the rolled paper as a living seed. When the radicle length was longer than the length of the seed, the seed was given a decision for the germinated seed group. When the radical length was shorter than the seed length, the seed was classified into the dead seed group.

Discriminant analysis

The spectra of the live soybean which germination percentage was 99.5% were classified into the live bean series, A99.5%LiveCal (n = 47). The spectra of the dead soybean which germination percentage was 6% were classified into the dead bean series, B6%DeadCal (n = 47). The library used to make a cluster calibration consisted of two series, A99.5%LiveCal and B6%DeadCal.

To make the database used for cluster analysis, the spectrum of the germinated soybean was classified into the series of living soybean, while the spectrum of the dead soybean which did not germinate was classified into the series of dead bean.

To make a qualitative discriminant model, the cluster calibration based on principal component analysis (PCA) was carried out on the calibration set spectra consisted of two series, the live bean and the dead bean series. PCA was used to reorder the spectral information by calculating a set of factors which best describe the variance between all calibration set spectra. The number of factors calculated was six in this study. To produce a cluster calibration model, a few factors which has smaller value of the correlation index was selected. The correlation index was a numerical guide designed to help to determine which factors were relevant for clustering, and the index was determined by stepping through the scores for each factor from the minimum to the maximum value.

The cluster analysis was carried out using software called Sesame ver.3.1 supplied by Bran+Luebbe Co.

Results and discussion

NIR spectrum of soybean

An example of the NIR spectrum of soybean is shown in Figure 1. The NIR absorption of water was observed at 1940, 1480 and 1200 nm. The absorption assigned to oil was observed at the ranges from 1653 to 1804 nm and from 2252 to 2395 nm.

Cluster analysis results using full range of spectrum data from 1000 to 2495 nm

Cluster analysis was carried out on the calibration library using full range of spectrum data from 1000 to 2495 nm (Figure 2). In Figure 2, the scores of factor 3 and 2 are plotted on the horizontal

and the vertical axes, respectively. The live beans could be distinguished from the dead beans clearly. The result shows the possibility of determination of the viability of a single grain of soybean using a near infrared spectroscopy.

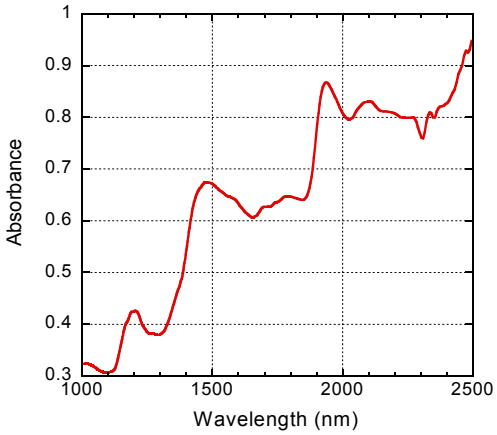

Figure 1. NIR spectrum of soybean

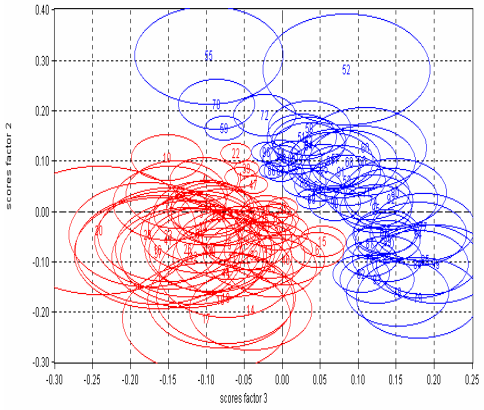

Figure 2. Results of cluster analysis using full range spectrum data of A99.5%LiveCal (left) and B6%DeadCal (right).

Effects of the spectral data ranges assigned to water and peptide bond on cluster analysis

Moisture, fat and protein contents of soybean have been measured using NIR.[3] The NIR absorption spectrum of water consists of five bands at 1940, 1450, 1190, 970 and 760 nm. The bands at 1450, 970 and 760 nm are the first, second and third overtones of O−H stretch, respectively. Those at 1940 and 1190 nm are combination bands involving O−H stretch and O−H bend. Cluster analysis was carried out on the calibration library using the spectrum data from 1449 to 1459 nm and from 1929 to 1943 nm. The live beans could not be distinguished from the dead beans (Figure 3). While, cluster analysis was carried out on the calibration library using the full range spectrum data except for the both data regions from 1449 to 1459 nm and from 1929 to 1943 nm. The live beans could be distinguished from the dead beans (Figure 4). The results said that the spectrum data assigned water was not significant to the analysis.

The NIR absorption spectrum of protein is observed at 2170 nm which is assigned to the peptide bond of protein.[5] Cluster analysis was carried out on the calibration library using the spectrum data from 2165 to 2176 nm. The range was assigned to peptide bond absorption. The live beans could not be distinguished from the dead beans (Figure 5). While, the analysis was carried out on the calibration library using the full range spectrum data except for the data region from 2165 to 2176 nm. The live beans could be distinguished from the dead beans (Figure 6). The results said that the spectrum data assigned peptide bond was not significant to the analysis.

Effects of the spectral data ranges assigned to oil on cluster analysis

Cluster analysis was carried out on the calibration library using the spectrum data from 1653 to 1804 nm or from 2252 to 2395 nm. These ranges are assigned to oil absorption. The live beans could be distinguished from the dead beans in both spectrum data regions, though several outsiders were observed (Figures 7 and 8). These results said that the spectrum data assigned oil were

significant to the analysis and the possibility of determination of the viability of a single grain of soybean using a near infrared spectroscopy.

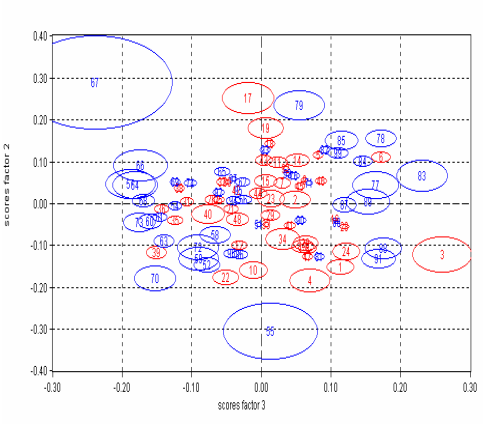

Figure 3. Result of cluster analysis using both spectrum data from 1449 to 1459 nm and from 1929 to 1943

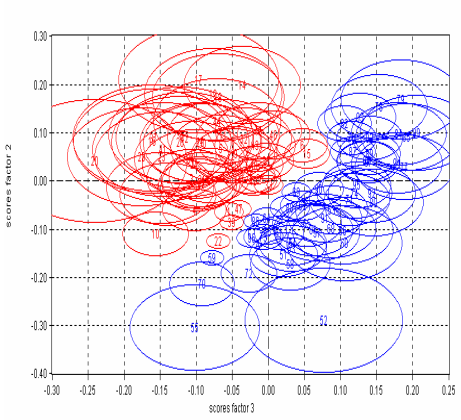

Figure 4. Result of cluster analysis using full range spectrum data except for the data from 1449 to 1459 and from 1929 to 1943 nm

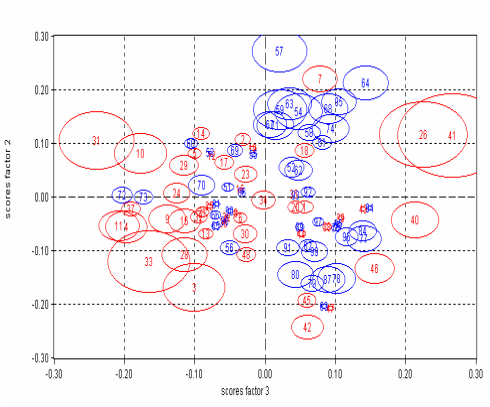

Figure 5. Result of cluster analysis using spectrum data from 2165 to 2176 nm.

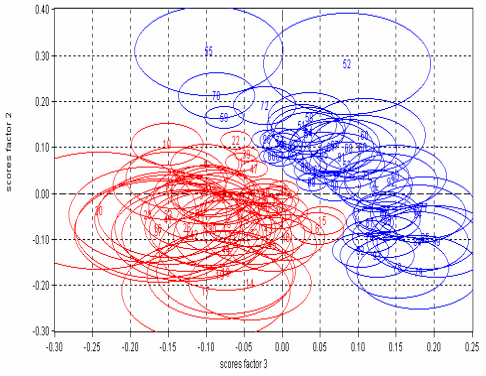

Figure 6. Result of cluster analysis using full range spectrum data except for the data from 2165 to 2176 nm

In conclusion, it might be possible to determine the viability of a single grain of soybean using a near infrared spectroscopy. The significant wavelength regions of the spectrum on cluster analysis depended on the absorption of the oil in soybean. Development of an automatic sorting machine of soybean using NIR will be studied in the future.

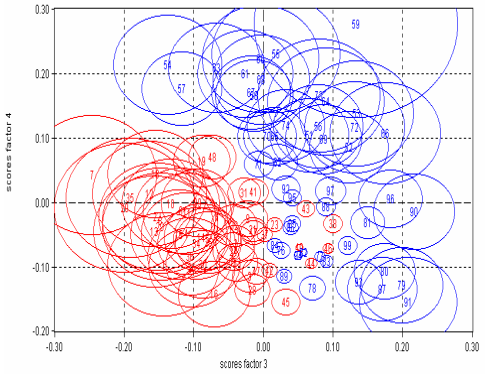

Figure 7. Result of cluster analysis using spectral data from 1653 to 1804

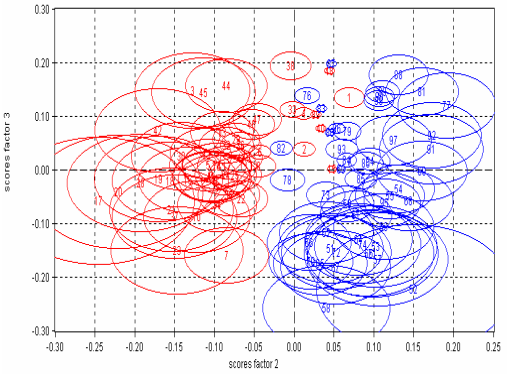

Figure 8. Result of cluster analysis using spectral data from 2252 to 2395

Acknowledgement

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