Non-destructive determination of protein content in a single kernel of wheat and soybean by near infrared spectroscopy

Hideyuki Abe, Toyoko Kusama and Sumio Kawano
National Food Research Institute, Tsukuba 305, Japan.

Mutsuo Iwamoto
Ministry of Agriculture, Forestry and Fisheries, Tokyo 100, Japan.

Introduction

Near infrared (NIR) spectroscopy as a non-destructive method for determining chemical components is successfully used in various fields of industry today. Especially, the remarkable development of NIR instruments and data treatment procedures (chemometrics) has contributed to the accurate determination of chemical components such as moisture, protein, lipid etc. However, in the case of the determination of protein content in a grain such as wheat and soybean, samples usually should be ground for measuring the NIR spectrum in the reflectance mode. Development of non-destructive methods for the determination of chemical components in a single kernel can provide a novel technique for the rapid selection of seed which is required for plant breeding. Also, separation of high quality grain from mixtures can produce value-added materials which meet end-user requirements for a food product. Recently, NIR spectrophotometers equipped with fiber optics have become commercially available which are useful for measuring NIR spectra of samples with irregular shapes such as fruit or a seed. In this study, we applied NIR spectroscopy with fiber optics to the non-destructive determination of protein content in a single kernel of wheat and soybean.

Materials and method

In this study, 90 wheat samples and 125 soybean samples cultivated in Japan were used. Wheat samples differed in variety, growing region or cultivated year and soybean samples differed in variety. Actual protein content in these samples was analyzed by an organic elemental analyzer (LECO FR-428) after drying for 16 hours in an oven at 135°C. We employed a nitrogen–protein conversion constant of 5.83 for wheat and 6.25 for soybean. Statistical results of protein content are shown in Table 1.

In a previous study, it was concluded that measurement of spectra by transmittance mode was more practical because reflectance from a grain was very weak. Therefore, the NIRSystems 6500 spectrometer with fiber optics was employed for measuring spectra in the transmittance mode. We specially designed a sample cell for the spectrometer (Figure 1).

Table 1. Statistical result of chemical analysis of protein content in a single kernel of wheat and soybean.

	Wheat		Soybean	
	CAL[a]	PRE[b]	CAL	PRE
Max.	16.95	15.71	38.46	36.68
Min.	6.36	6.83	25.02	26.96
Average	9.49	9.41	31.24	31.28
S.D.	2.34	2.23	2.50	2.23
Samples	45	45	63	62

[a]Calibration sample.
[b]Prediction sample.

NIR spectra measurement was performed in the wavelength region from 400 nm to 1100 nm. Each sample of wheat and soybean was irradiated and spectra measured from different directions to estimate the influence of the shape of samples (Figure 2). Spectral data were transferred to a UNIX workstation to calculate calibration equations by multiple linear regression. Calibration equations were developed individually using the spectra obtained in each direction.

Result and discussion

Typical NIR spectra of wheat and soybean measured in the transmittance mode at the wavelength region from 800 nm to 1100 nm are shown in Figure 3. Broad absorption bands appear at 916 nm, 984 nm and 1016 nm. Many absorption bands assigned to different molecular vibra-

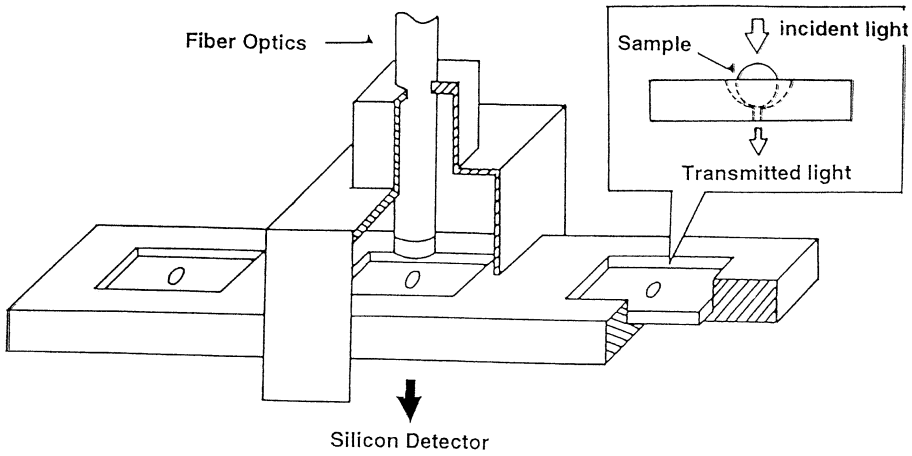

Figure 1. Representation of sample cell for a single grain.

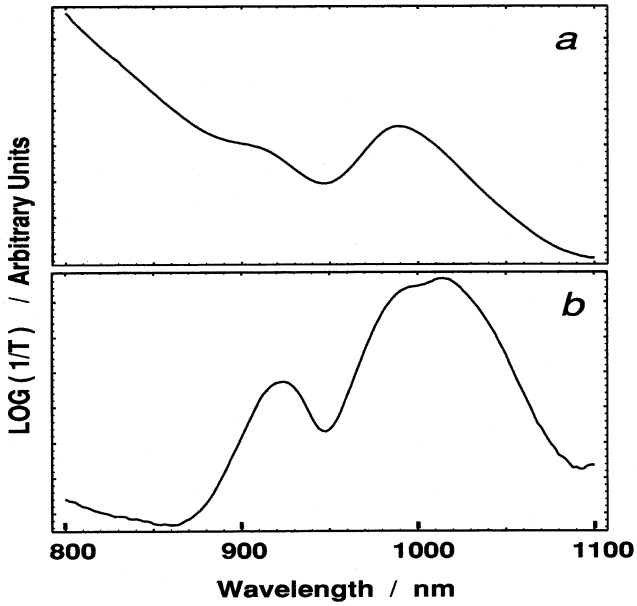

Figure 2. NIR spectra of a single kernel of (a) wheat and (b) soybean measured by transmittance mode with fiber optics.

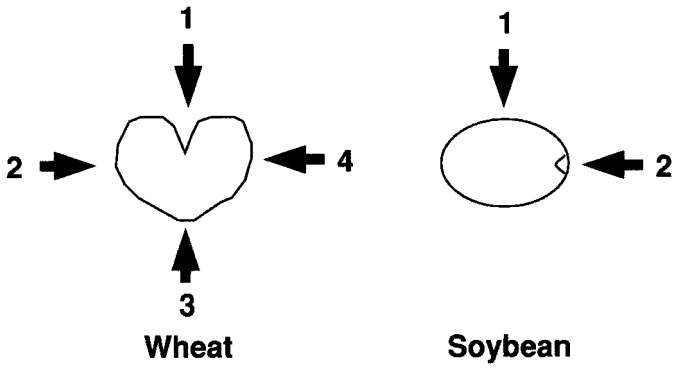

Figure 3. Direction of incident light. Transmitted light is detected on the opposite side of the irradiated surface of the sample.

tions are overlapped in this region, however, absorption bands at 916 nm, 984 nm and 1016 nm are generally assigned to the stretching vibration of the CH group, OH group and NH group, respectively.

Statistical results of prediction error for protein content are listed in Table 2 and Table 3. It is concluded that the standard error of prediction (*SEP*) is affected by the measurement direction of the samples. However, the calibration equation developed by all the calibration samples is applicable to all of the validation samples measured from different directions, and using the mean

Table 2. Prediction error of protein content in a single kernel of wheat by NIR spectroscopy.

Direction	1	2	3	4	Averaged[f]
Equation 1[a]	1.09/0.32[g]	1.84/–0.47	1.11/0.26	1.80/–0.49	0.97/–0.10
Equation 2[b]	1.13/0.47	1.26/–0.19	0.98/0.02	1.16/0.33	0.81/0.16
Equation 3[c]	1.69/0.92	1.39/0.22	1.25/0.19	1.42/0.47	0.95/0.45
Equation 4[d]	1.89/0.47	1.45/0.05	2.66/1.86	1.42/–0.22	1.62/0.54
Equation 5[e]	1.05/0.28	1.25/–0.45	1.01/0.08	1.04/0.02	0.77/0.02

Selected wavelengths:
[a]800, 814, 974, 988, 1020, 1034, 1038 and 1066 nm.
[b]1004, 1022 and 1070 nm.
[c]994, 996, 1002, 1016, 1022 and 1066 nm.
[d]810 and 820 nm.
[e]1002, 1022 and 1070 nm.
[f]Using averaged NIR value on the all direction.
[g]*SEP* (%)/BIAS.

Table 3. Prediction error of protein content in a single kernel of soybean by NIR spectroscopy.

Direction	1	2	Averaged[d]
Equation 6[a]	0.74/–0.09[e]	1.98/–1.13	1.12/–0.61
Equation 7[b]	1.23/–0.46	1.19/0.10	1.06/–0.18
Equation 8[c]	0.68/–0.18	0.95/0.03	0.67/–0.07

Selected wavelength:
[a]812, 862, 874, 892, 904, 906, 914, 964, 1004, 1010 and 1070 nm.
[b]942, 950, 1000, 1018, 1026, 1040, 1044 and 1060 nm.
[c]806, 836, 896, 904, 926, 992, 1018, 1056 and 1062 nm.
[d]Using averaged NIR value in all directions.
[e]*SEP*(%)/BIAS.

value of each sample as the predicted value, the *SEP* is improved to a value of 0.77% for wheat and 0.67% for soybean. This result indicates that spectra measurement from different directions and calculation of the mean value of predicted value is of importance for a sample of irregular shape such as wheat.

Conclusion

We specially designed a sample cell for a single kernel of wheat and soybean and measured NIR spectra in the wavelength region from 400 nm to 1100 nm by transmittance mode with fiber optics. The *SEP* of protein content in wheat and soybean were 0.77% and 0.67%, respectively. This result is considered to be insufficient to be of practical use. It is suggested that the NIR method has the possibility to determine protein content in a single kernel of wheat and soybean, however, further experiment is necessary to improve the accuracy for practical use, especially in the selection of seeds.