Non-destructive analysis of anthocyanin content and antioxidant activity in single purple corn kernel by near-infrared reflectance spectroscopy

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Introduction

Purple corn has an added benefit because it contains anthocyanin, a powerful antioxidant, in its cob, kernel, and leaf-stems, owing to which, it may offer additional health benefits when used as a livestock feed (Hosoda et al., 2012). Japanese corn breeding units have been developing a highly digestible purple corn, high in anthocyanin content. However, the conventional method for estimating physiological functions is labor intensive. Near-infrared reflectance (NIR) spectroscopy is one of the most powerful analytical tools used in the fields of agriculture and food research. We examined the feasibility of using NIR spectroscopy for the estimation of anthocyanin contents and 1,1-diphenyl-w-picrylhydrazyl (DPPH) radical-scavenging activity in the cob, kernel, and leaf-stems of purple corn. The results for the coefficient of determination for validation (R^2v) and standard deviation/square error of validation values (RPD), were, respectively, as follows: cob anthocyanin contents, 0.91 and 2.4; kernel anthocyanin contents, 0.64 and 1.3; leaf-stem anthocyanin contents, 0.95 and 5.6.; cob DPPH radical-scavenging activity, 0.93 and 2.6; kernel DPPH radical-scavenging activity, 0.84 and 1.9; leaf-steam DPPH radical-scavenging activity, 0.98 and 3.1 (Eguchi et al., 2011). The RPD is a useful measure for evaluating calibration (Williams 2001). A calibration that produces a high RPD (>2.4) and a high R^2_v (>0.81) is beneficial for screening. These values demonstrated the usefulness of NIRS screening in the breeding process. Powdered cob and leaf-stem showed high R^2_v and RPD values, which demonstrate the utility of NIR spectroscopy screening for breeding. However, using powdered kernel, low R^2_v and RPD were obtained, demonstrating in this case the appropriateness of NIR spectroscopy screening. If a calibration equation for determining anthocyanin content and DPPH radical-scavenging activity could be established using NIR spectroscopy nondestructively in single corn kernels, the analysis could then be performed in the field.

Material and Method

1. Plant material

One inbred line ear and six F_1 hybrid lines ears were selected at the maturation stage at the (National Agriculture and Food Research Organization of Livestock and Grassland Science, Tochigi, Japan) in 2014. One hundred and twenty corn kernels were collected from these ears. These samples were first pulverized by using a crushing mill. Prior to the chemical analysis, the powdered samples were individually treated with 1 mL of water-concentrated trifluoroacetic acid (99/1, v/v) for 24 hr at room temperature. The seed powder final concentration was 20 mg/mL. These extracts were used to measure anthocyanin contents. After anthocyanin measurements, the extracts were dried, and treated with methanol. The final concentration was 20 mg/mL. These extract were used for measuring the DPPH radical-scavenging activity.

2. Chemical measurement

Anthocyanin contents were calculated using a calibration curve for cyanidin-3-glucoside at 520 nm and expressed as the Cy3Glc equivalent per gram of dried weight. The DPPH radical-scavenging activity was measured using a previously described method (Oki et al. 2003). Briefly, 50 µL of a 20% ethanol solution and 50 µL of a 200 mM MES buffer were added to 50 µL of a sample solution into a well of a 96-wells microplate. The sample solution was prepared by diluting the purple corn extract with a methanol solution. The reaction was initiated by the addition of 50 µL of 500 µM DPPH in ethanol. After the reaction mixture was allowed to stand for 20 min at room temperature, its absorbance at 520 nm was measured. The DPPH radical-scavenging was estimated by the decrease in absorbance at 520 nm and expressed as the trolox equivalent per gram of dried weight by using a standard curve of trolox. The anthocyanin content and DPPH radical-scavenging activity of the samples are summarized in Table1.

3. Near-infrared reflectance spectroscopic measurements

A SpectraStar2400 instrument (BL-Tec K.K., Osaka, Japan) was used to measure the NIR spectra in the wavelength range from 1200 to 2400 nm at 1-nm intervals.

Figure 1 shows the original cup for a single kernel. For the calibration, measurements were completed in duplicate, and the average of the two measurements was used for subsequent statistical analysis (PLSR). Each sample type was divided in two sets: a calibration set (n = 90) and validation set (n = 30).

4. Statistical analysis

The partial least square regression (PLSR) of the NIR spectra data with the chemical data was carried out on the calibration set using the Calibration Workshop 2.1 (Sensolobic GmbH., Germany). The spectra were explored by second-derivative maths. In this case, the conditions to obtain the derivatives were as follows: segment 6 nm, gap 2 nm or segment 4 nm, and gap 4 nm. The validation of the calibration equation was carried out using the validation set.

Result and Discussion

In this study, the calibration of anthocyanin contents and DPPH radical-scavenging activity provided the lowest SEP (3.04 µmol Cy3Glc eq/g D.W. And 1.66 µmol Trolox eq/g D.W.). RPD and R^2_v were as follows: anthocyanin content, 0.84 and 2.5; DPPH radical-scavenging activity, 0.87 and 2.7 (Table 2). Previous work, using the powder of purple corn kernels, the calibration provided low RPD (<2.3) and R^2_v (<0.81) (Eguchi et al., 2011). However, using single purple corn kernels, the calibration provide high RPD (>2.4) and R^2_v (>0.81). These different results were shown because near-infrared radiation transmutes under 2–3 mm of radiation target. In other words, near-infrared radiation transmutes into the pericarp and the aleurone layer, which accumulate abundant anthocyanins. Correlation of anthocyanin content and DPPH radical-scavenging activity was 0.90 ($p < 0.01$, $n = 120$), Thus, the results seem to highlight the high precision of the calibration curve of DPPH radical-scavenging activity. These outcomes indicate that anthocyanin content and DPPH radical-scavenging activity of single purple corn kernels could be measured nondestructively using NIR spectroscopy, (Fig. 2). Figure 3A shows the anthocyanin content regression coefficient vector derived using PLSR. Anthocyanins have the 1st overtone of OH at a max wavelength of 1663 nm. Figure 3B shows the DPPH radical-scavenging activity regression coefficient vector derived using PLSR. DPPH radical-scavenging activity has the 1st overtone of OH at max wavelength 1665nm. The results show that DPPH radical-scavenging activity reflects the anthocyanin contents in purple corn kernel.

Figure1. NIR spectrophotometer equipped with a single kernel adapter (dent φ=2.7cm).

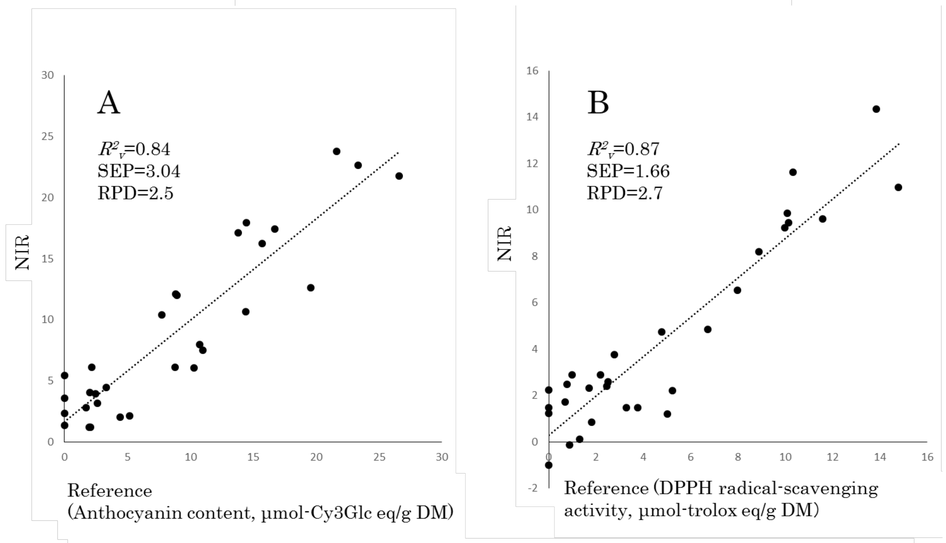

Figure2. Relation between laboratory-determined and NIR spectroscopy-predicted values for anthocyanin content and DPPH radical-scavenging activity of single purple corn kernel.

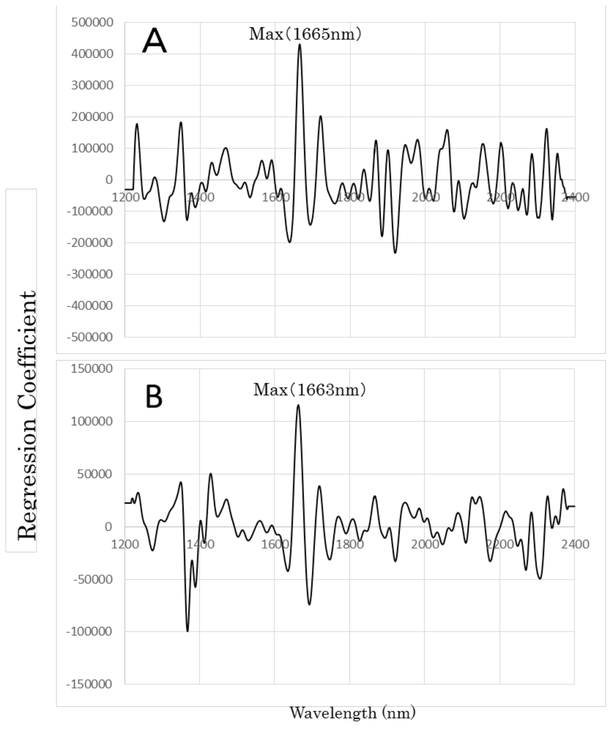

Figure3. (A: anthocyanin content, B: DPPH radical scavenging activity) regression coefficient vectors derived using PLSR. The max wavelength corresponding to major constituents in corn kernel is shown.

Table1. Analytical data of calibration and validation sets.

	Calibration set (n=90)				Validation set (n=30)			
	Max	Min	Mean	SD	Max	Min	Mean	SD
Antocyanin contetnts (µmol-Cy3Glc eq/gDM)	27.1	0.0	5.5	7.6	26.6	0.0	8.4	7.7
DPPH radical-scavenging act (µmol-Trolox eq µmol/gDM)	15.3	0.0	3.7	3.7	14.8	0.0	4.9	4.4

Max:maximum; Min:minimum; SD:standard deviation

Table2. PLR results for determing anthocyanin content and DPPH radical-scavenging activity of single purple corn kernel.

		Calibration			Validation		
	math	nF	SEC	R^2_C	SEP	R^2_v	RPD
Antocyanin contetnts (µmol-Cy3Glc eq/gDM)	2Der	15	2.44	0.89	3.04	0.84	2.5
DPPH radical-scavenging activity (µmol-Trolox eq µmol/gDM)	2Der +Smoothing	17	1.22	0.89	1.66	0.87	2.7

2Der: Second derivative; nF: Factor; SEC: the standard error of calibration
R^2_c: the correlation coefficient of calibration; R^2_v: the correlation coefficient of validation
RPD: Ratio of the standard error of performance to the standard deviation of the reference data. High RPD (>2.4) is benefitical for screening.