

Determination of Cyanidin-3-Glucoside in Black Soybeans

Anthocyanins, essential factor to fix the color tone of flowers and fruits, are the generic name of the glycosides which have anthocyanidins as aglycones. The color tone of anthocyanins varies greatly according to the pH. It is known that many types of anthocyanins exist widely throughout higher plants, and recently they are receiving much attention due to their antioxidative properties.

Among these, cyanidin-3-glucoside, the glucose glycoside of cyanidin, is said to account for more than 90 % of the anthocyanidins present in black soybeans. Here we introduce an analysis of cyanidin-3-glucoside using the Prominence SPD-M20A photodiode array detector.

■ Analysis of Standard Solution

Fig.1 shows the structural formula of cyanidin-3-glucoside, and Fig.2 shows its absorption spectrum obtained under acidic conditions. It is clear that absorption is greatest in the vicinity of 280 nm and 520 nm.

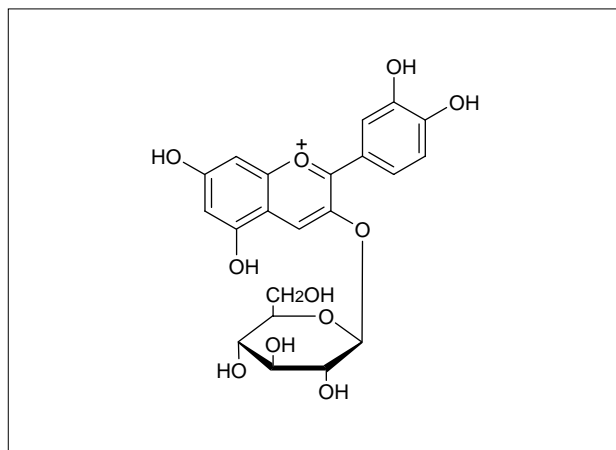


Fig.1 Structure of Cyanidin-3-Glucoside

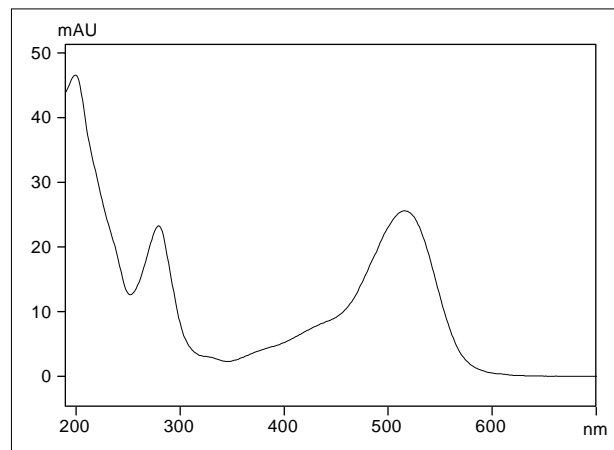


Fig.2 Spectrum of Cyanidin-3-Glucoside

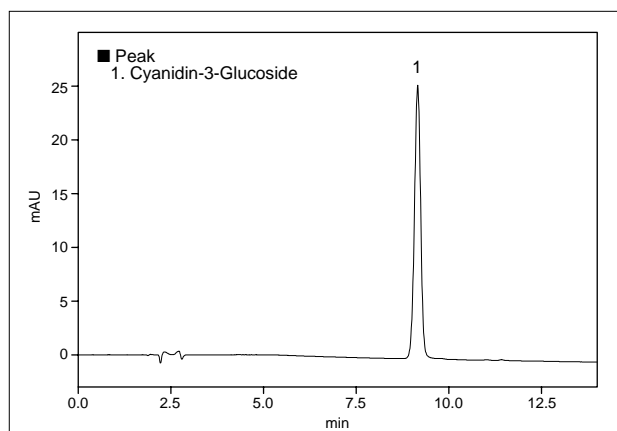


Fig.3 Chromatogram of Cyanidin-3-Glucoside (20 mg/L, 10 μ L Inj.)

Fig.3 shows an example of a standard substance analysis. To apply it to an analysis of the food sample, we used the gradient elution because of washing the column. The analytical conditions are shown in Table 1.

Table 1 Analytical Conditions

Column	: Shim-pack VP-ODS (150 mmL. \times 4.6 mmI.D.)	
Guard Column	: Shim-pack GVP-ODS (10 mmL. \times 4.6 mmI.D.)	
Mobile Phase	: A : 10 mM (Sodium) phosphate buffer (pH 2.6) B : Acetonitrile	
Initial B.Conc	= 5 %	
	Time (min)	B.Conc (%)
	10.00	30
	15.00	100
	20.00	100
	20.01	5
	35.00	STOP
Flow Rate	: 1.0 mL/min	
Column Temp.	: 40 $^{\circ}$ C	
Detection	: SPD-M20A at 520 nm (Slit width : 8 nm)	

■ Linearity

Fig.4 shows the calibration curve of cyanidin-3-glucoside which concentration range is from 0.1 to 20 mg/L (10 μ L injections) detected at 520 nm. Excellent linearity was obtained, with $R^2 = 0.9999$. Furthermore, S/N was about 11 with a 0.1 mg/L standard solution.

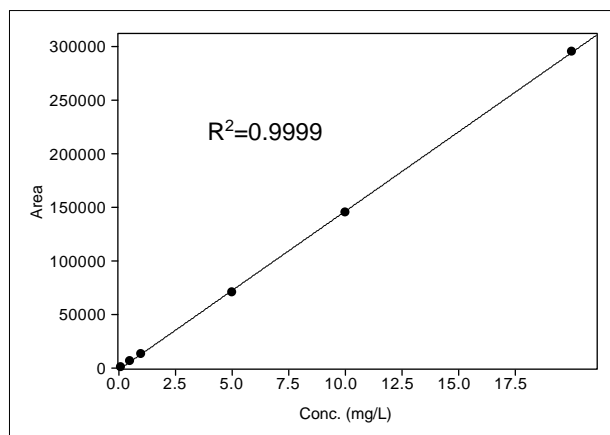


Fig.4 Linearity (0.1 to 20 mg/L, 10 μ L Inj.)

■ Analysis of Black Soybeans

Fig.5 shows the chromatogram of commercial black soybean extract solution obtained at two wavelengths. After pulverizing the black soybeans, 5 mL of methanol containing 1 % hydrochloric acid was added to 1 g of the ground powder. Cyanidin-3-Glucoside was extracted using ultrasonication, and centrifugation and filtering to the extract were performed. And then,

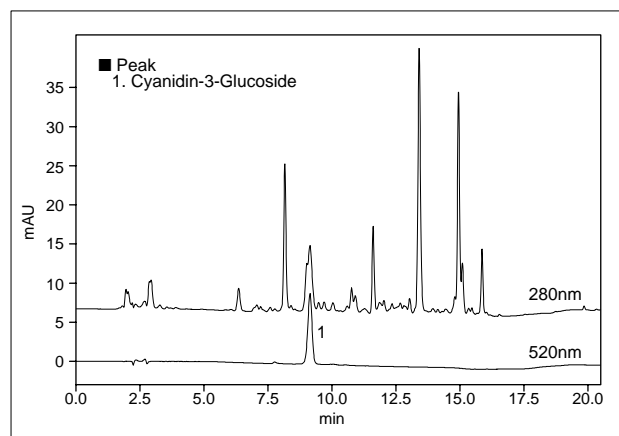


Fig.5 Chromatograms of Black Soybean Extract (10 μ L Inj.)

mobile phase A was added to make a 10-fold dilution. Fig.6 shows the spectra of the cyanidin-3-glucoside in the black soybean extract solution and the standard solution. A contour plot of the black soybean extract solution is shown in Fig.7, and a 3-D plot is shown in Fig.8.

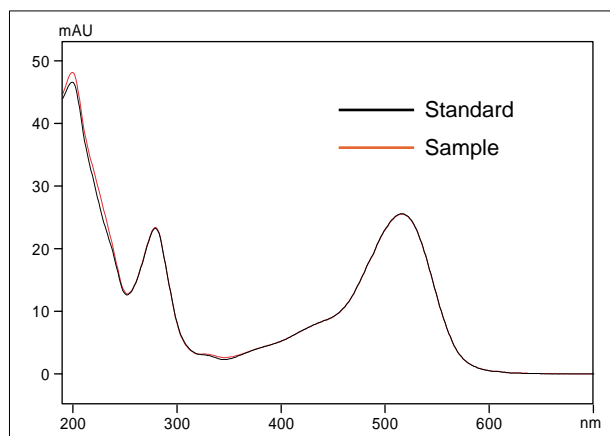


Fig.6 Spectra of Cyanidin-3-Glucoside in Black Soybean Extract and Standard Compound

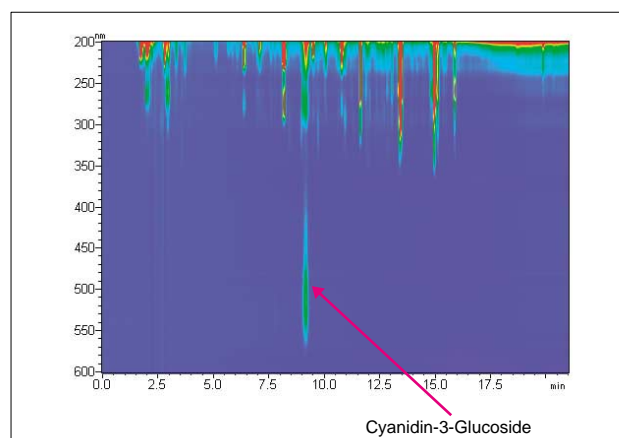


Fig.7 Contour Plot of Black Soybean Extract

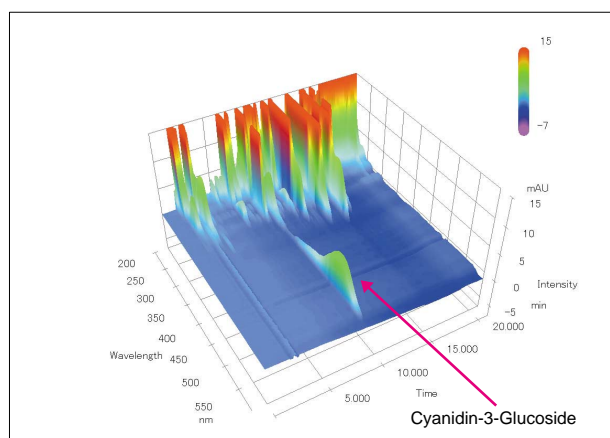


Fig.8 3-D Plot of Black Soybean Extract

NOTES:

*This Application News has been produced and edited using information that was available when the data was acquired for each article. This Application News is subject to revision without prior notice.



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