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High Performance Liquid Chromatography Nexera™

Application News

Quantitative Analysis of Anthocyanins in Tea Leaves and Barley

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User benefit

◆ Four anthocyanins can be analyzed in 25 min.

◆ Since quantified in glycoside form not in aglycone equivalent, it is possible to analyze only by extraction without hydrolysis.

◆ Conventional HPLC systems can be used with this developed method.

■ Introduction

In collaboration with the National Agriculture and Food Research Organization,

Shimadzu Corporation has been developing a simple, quick and accurate method of analyzing functional components in agricultural and food products. In this report, a quantitative method of anthocyanins was developed and the content in two kind of tea leaves and one kind of barley was showed.

Anthocyanins are pigments which appear red, purple or blue. Anthocyanins are contained in plants in the form of glycosides with sugars or sugar chains bound together. In this report, 4 anthocyanins shown in Table 1 were analyzed as target compounds.

Table 1 Target Compounds

Compound
Delphinidin-3-galactoside
Delphinidin-3-glucoside
Cyanidin-3-galactoside
Chrysanthemin-3-glucoside

■ Analytical Conditions

The analytical conditions were determined with reference to existing methods.[1),2),3)]. A Shim-pack™ GIST HP (150 mm x 4.6 mm I.D 3 μm) column as used for analysis, and the target anthocyanins were separated in 25 min. The analytical conditions are shown in Table 2.

Table 2 Analytical Conditions

System	: Nexera X3
Column	: Shim-pack GIST-HP C18[*1]
	(150 mm × 4.6 mm I.D., 3 μm)
Mobile Phases	: A) 2 % formic acid in H_2O
	B) 2 % formic acid in H_2O /Acetonitrile=1/1 (v/v)
Gradient Program	: B conc. 15 % (0.00-3.00 min) - 30 % (15.00 min)
	-100 % (15.01-20.00 min) - 15 % (20.01-25.00 min)
Flow rate	: 1.0 mL/min (Maximum pressure: 16 MPa)
Column Temp.	: 40° C
Injection Volume	: 10 μL
Detection	: 520 nm （W lamp）

＊1 P/N：227-30041-05

■ Sample Pretreatment

The extraction was performed following the conditions(Fig.1) determined with reference to existing methods[1) 2)].
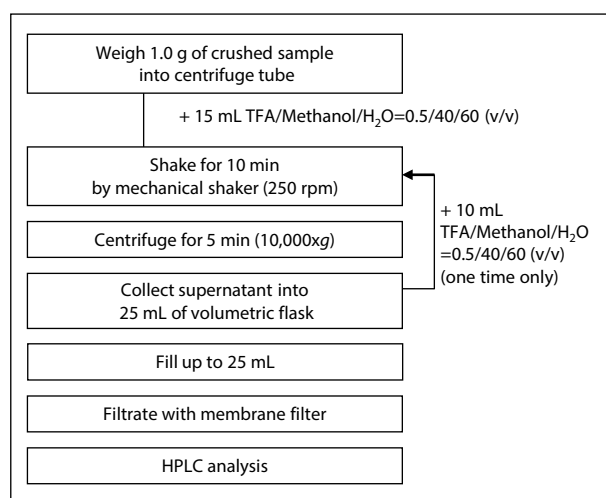

Fig. 1 Pretreatment Workflow

■ Analysis Results of Standard Samples

The linearities were confirmed using standards analysis.

Fig. 2 shows the calibration curves, and Fig. 3 shows a representative 10 μg/mL chromatogram.

Table 3 shows the dynamic range and the coefficients of determination. Good linearities were obtained with a coefficient of determination (R^2) ≥ 0.999 for all compounds.

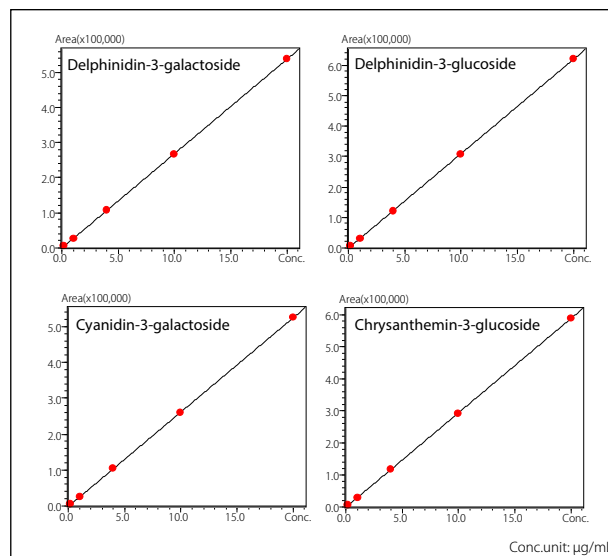Fig. 2 Calibration Curves

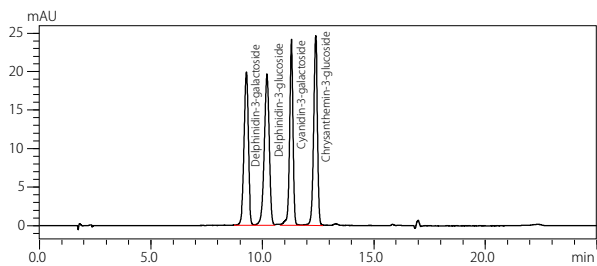Fig. 3   Chromatogram of 10 µg/mL Standard Sample

Table 3 Linear range and Coefficient of Determination (R²)

Compound	Linear range (µg/mL)			Coefficient of determination (R²)
Delphinidin-3-galactoside	0.20	-	20	0.9999
Delphinidin-3-glucoside	0.20	-	20	0.9999
Cyanidin-3-galactoside	0.20	-	20	0.9999
Chrysanthemin-3-glucoside	0.20	-	20	0.9999

■ Repeatability Test Results of Tea Leaf and Barley Extracts

Seven extracts were prepared from the tea leaf and barley samples, and a repeatability test was performed to confirm the validity of the method. For tea leaves, the Sunrouge variety, which has a high anthocyanin concentration, was used.

For barley, a sample composed of a mixture of different kinds of barley with added anthocyanins at a concentration of 0.01 mg/g was used. Tables 4 and 5 show the results.

Table 4   Repeatability Test Results of Tea Leaves (n=7)

Compound	Repeatability (%RSD)
Delphinidin-3-galactoside	1.52 %
Delphinidin-3-glucoside	2.03 %
Cyanidin-3-galactoside	1.10 %
Chrysanthemin-3-glucoside	1.08 %

Table 5 Repeatability Test Results of Barleys (n=7)

Compound	Repeatability (%RSD)
Delphinidin-3-galactoside	3.40 %
Delphinidin-3-glucoside	3.77 %
Cyanidin-3-galactoside	4.72 %
Chrysanthemin-3-glucoside	3.34 %

■ Quantitative Results for Tea Leaves and Barleys

The extracts of two varieties of tea (Yabukita, Sunrouge) and one variety of barley (Daishimochi) were analyzed to determine their anthocyanin content. Fig. 4 shows the chromatograms, and Table 6 shows the calculated content of each anthocyanin per 100 g of sample.

Table 6   Anthocyanin Content in Tea Leaf and Barley

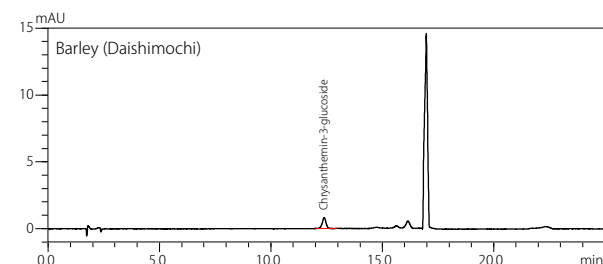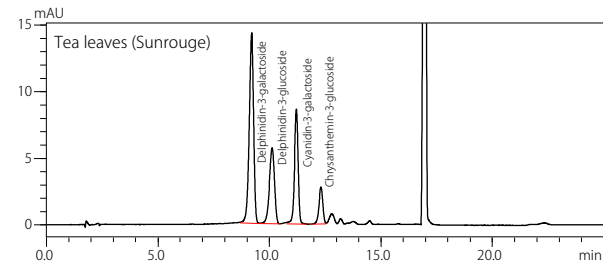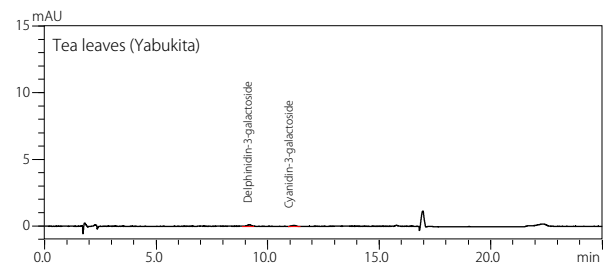| Compound	Content （mg/100g）		
	Tea leaves (Yabuita)	Tea leaves (Sunrouge)	Barleys (Daishimochi)
Delphinidin-3-galactoside	< LLOQ	0.18758	n.d.
Delphinidin-3-glucoside	n.d.	0.07461	n.d.
Cyanidin-3-galactoside	< LLOQ	0.09776	n.d.
Chrysanthemin-3-glucoside	n.d.	0.03070	0.00865

n.d.：not detected

Fig. 4 Chromatograms of Tea Leaves and Barleys Extract

■ Conclusion

- Simultaneous analysis of four anthocyanins was performed using the Nexera series.
- The content of anthocyanins in two varieties of tea and one variety of barley was determined using the developed method.

<References>
1) Oki, T., Analysis of anthocyanins in purple sweet potato, Food Functionality Evaluation Manual Vol.I (Revised 2nd Edition) (http://fmric.or.jp/ffd/kinousei-hyoka.html)
2) Oki, T., Analysis of anthocyanins in purple sweet potato, Food Functionality Evaluation Manual Vol.Ⅳ (http://fmric.or.jp/ffd/kinousei-hyoka4.html)
3) Masada, S., Uchiyama, N., Goda, Y., Hakamatsuka, T., An analysis of anthocyanins in "Foods with Functional Claims" containing bilberry extracts, Japanese Journal of Food Chemistry and Safety (JJFCS), Vol. 24(1), 32-38(2017)

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