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Application News

No. L581

High Performance Liquid Chromatography

Quantitative Analysis of Flavonols in Tea Leaves

In collaboration with the National Agriculture and Food Research Organization,

NARO
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. This report introduces a quantitative method for flavonols analysis in tea leaves and presents the results obtained in two kinds of them.

Flavonols, a kind of polyphenols, are classified into flavonoids. Generally, to determine the content of flavonols, the glycosides are hydrolyzed to provid only the aglycone form rate. In this report, an analysis method of flavonols shown in Table 1 was developed to determine the contents of glycosides and aglycone without hydrolysis.

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Table 1 Target Compounds

Compound
Kaempferol
Quercetin
Isoquercitrin (quercetin 3-glucoside)
Hyperoside (quercetin 3-galactoside)
Rutin (quercetin 3-rutinoside)
Myricetin
Myricetin 3-glucoside
Myricetin 3-galactoside

Sample Pretreatment

The extraction was performed following the conditions determined in the reference of pre-existing method[1]. The workflow is showed in Fig.1. The pretreatment was conducted by extracting crushed tea leaves with 80% MeOH solution and a dilution by 10 times in water.

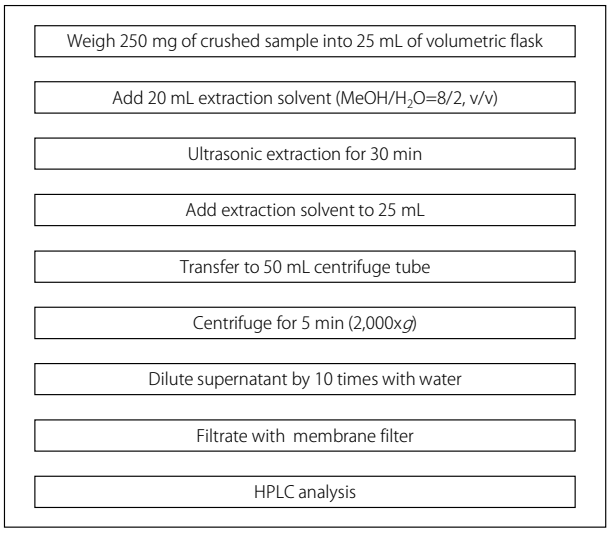

Fig. 1 Pretreatment Workflow

Analytical Conditions

The analytical conditions were determined in the reference of the pre-existing method[1), 2)].The analytical conditions are shown in Table 2.

Table 2 Analytical Conditions

System	:	Nexera™ X3
Column	:	Shim-pack™ GIST C18 (150 mm × 4.6 mm I.D., 3 µm P/N：227-30011-07)
Mobile phases	:	A) 0.1% Formic acid in H_2O B) Acetonitrile
Gradient Program	:	B conc. 15% (0-14.00 min) - 95% (22.01-24.00 min) -15% (24.01-30.00 min)
Flow rate	:	1.0 mL/min
Column Temp.	:	40 °C
Injection volume	:	10 µL
Detection	:	UV 370 nm

Analysis Results of Standards

The linearities were determined by the standards analysis. Fig. 2 shows a representative chromatogram and Table 3 shows the dynamic range and the coefficients of determination. Good linearities were obtained with a coefficient of determination (R^2) ≥ 0.997 for all compounds.

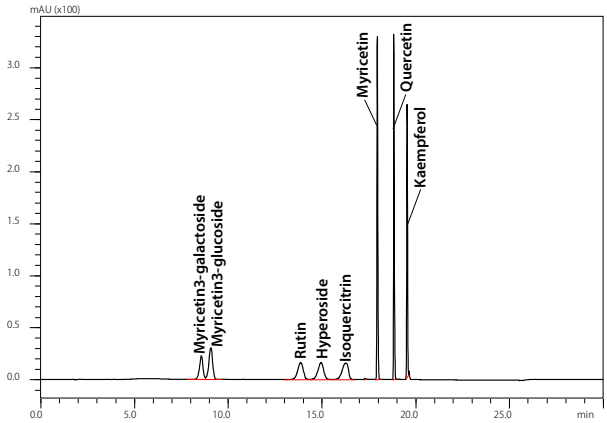

Fig. 2 Chromatogram of Standard Sample

Table 3 Linear range and Coefficient of determination (R^2)

Compound	Linear range (µg/mL)			Coefficient of Determination (R^2)
Kaempferol	0.1	-	20	0.9979
Quercetin	0.1	-	2	0.9992
Isoquercitrin	0.1	-	2	0.9993
Hyperoside	0.1	-	20	0.9996
Rutin	0.1	-	20	0.9998
Myricetin	0.1	-	20	0.9978
Myricetin3-glucoside	0.1	-	20	0.9998
Myricetin3-galactoside	0.1	-	20	0.9999

Repeatability Test Results of Tea Leaf Extracts

Seven extracts were prepared from one kind of tea (benifuuki) and repeatability test was performed to confirm validity. Table 4 shows the results.

Table 4 Repeatability Test Results (n=7)

Compound	Repeatability (%RSD)
Kaempferol	- (< LLOQ)
Quercetin	- (< LLOQ)
Isoquercitrin	0.66
Hyperoside	3.33
Rutin	- (< LLOQ)
Myricetin	1.19
Myricetin3-glucoside	2.74
Myricetin3-galactoside	2.79

Quantitative Results for Tea Leaves

The extracts of two kinds of tea (Yabukita, Benifuuki) were analyzed to determine the content of flavonols. Fig. 3 shows the chromatograms and Table 5 shows the calculated content of each flavonol in tea leaves.

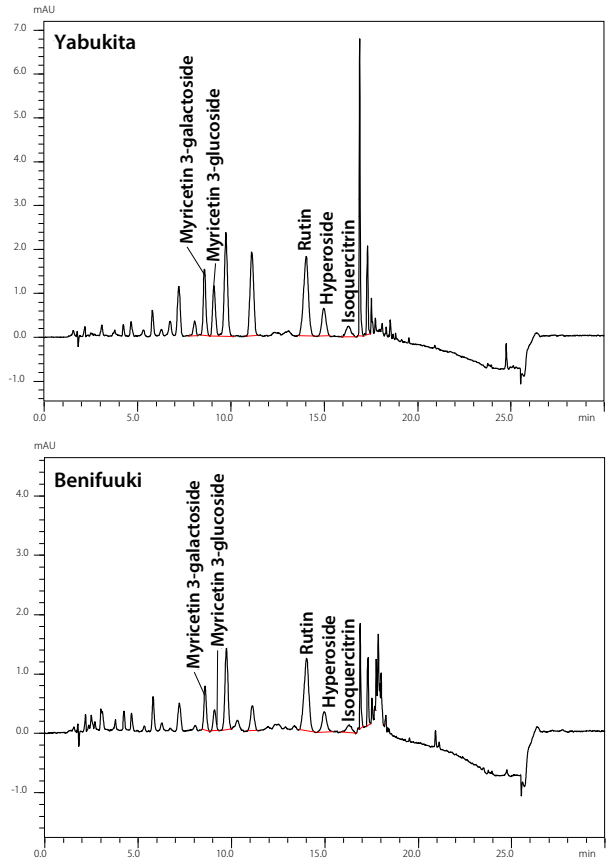

Fig. 3 Chromatograms of Tea Leaf Extracts

Table 5 Flavonol Content in Tea Leaves

Compound	Content (mg/g)	
	Yabukita	Benifuuki
Kaempferol	< LLOQ	< LLOQ
Quercetin	< LLOQ	< LLOQ
Isoquercitrin	0.252	0.131
Hyperoside	0.572	0.359
Rutin	1.975	1.348
Myricetin	n.d. *1	< LLOQ
Myricetin3-glucoside	0.575	0.182
Myricetin3-galactoside	1.006	0.501

*1 Not detected

Conclusion

- Using the Nexera series, simultaneous analysis of flavonols was performed.
- The flavonols quantification results show a difference in content depending on the kind of tea leaves.

<References>
1) Monobe et al. Quercetin Glycosides-rich Tea Cultivars (Camellia sinensis L.) in Japan. Food Science and Technology Research. 2015, 21 (3), p.333-340.
2) Nobuya Shirai. Assay of Flavonol Contents in Tea Leaves and Infusions. Nippon Shokuhin Kagaku Kogaku Kaishi. 2018, 65(7), p. 357-362.

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