

Application News

High Performance Liquid Chromatography

Quantitative Analysis of Flavonols in Tea Leaves

No. L581

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. 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 method¹⁾. 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.

| Weigh 250 mg of crushed sample into 25 mL of volumetric flask Add 20 mL extraction solvent (MeOH/H ₂ O=8/2, v/v) Ultrasonic extraction for 30 min Add extraction solvent to 25 mL Transfer to 50 mL centrifuge tube Centrifuge for 5 min (2,000xg) Dilute supernatant by 10 times with water Filtrate with membrane filter HPLC analysis | |
|---|---|
| Ultrasonic extraction for 30 min Add extraction solvent to 25 mL Transfer to 50 mL centrifuge tube Centrifuge for 5 min (2,000xg) Dilute supernatant by 10 times with water Filtrate with membrane filter | Weigh 250 mg of crushed sample into 25 mL of volumetric flask |
| Ultrasonic extraction for 30 min Add extraction solvent to 25 mL Transfer to 50 mL centrifuge tube Centrifuge for 5 min (2,000xg) Dilute supernatant by 10 times with water Filtrate with membrane filter | |
| Add extraction solvent to 25 mL Transfer to 50 mL centrifuge tube Centrifuge for 5 min (2,000xg) Dilute supernatant by 10 times with water Filtrate with membrane filter | Add 20 mL extraction solvent (MeOH/H ₂ O=8/2, v/v) |
| Add extraction solvent to 25 mL Transfer to 50 mL centrifuge tube Centrifuge for 5 min (2,000xg) Dilute supernatant by 10 times with water Filtrate with membrane filter | |
| Transfer to 50 mL centrifuge tube Centrifuge for 5 min (2,000xg) Dilute supernatant by 10 times with water Filtrate with membrane filter | Ultrasonic extraction for 30 min |
| Transfer to 50 mL centrifuge tube Centrifuge for 5 min (2,000xg) Dilute supernatant by 10 times with water Filtrate with membrane filter | |
| Centrifuge for 5 min (2,000xg) Dilute supernatant by 10 times with water Filtrate with membrane filter | Add extraction solvent to 25 mL |
| Centrifuge for 5 min (2,000xg) Dilute supernatant by 10 times with water Filtrate with membrane filter | |
| Dilute supernatant by 10 times with water Filtrate with membrane filter | Transfer to 50 mL centrifuge tube |
| Dilute supernatant by 10 times with water Filtrate with membrane filter | <u> </u> |
| Filtrate with membrane filter | Centrifuge for 5 min (2,000x <i>q</i>) |
| Filtrate with membrane filter | |
| | Dilute supernatant by 10 times with water |
| | , , |
| HPLC analysis | Filtrate with membrane filter |
| HPLC analysis | |
| 22 d.d.y515 | HPI C analysis |
| | unally 515 |

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 |
| Mobile phases | (150 mm × 4.6 mm l.D., 3 μm P/N ÷ 227-30011-07) : A) 0.1% Formic acid in H ₂ O B) Acetonitrile |
| Gradient | : B conc. 15% (0-14.00 min) - 95% (22.01-24.00 min) |
| Program | -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) \geq 0.997 for all compounds.

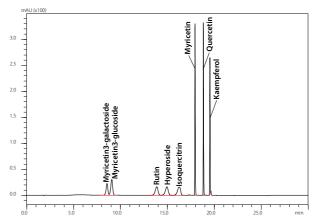


Fig. 2 Chromatogram of Standard Sample

Table 3 Linear range and Coefficient of determination (R2)

| Compound | Linear range (μg/mL) | | (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 |
| Myricetin 3-galactoside | 0.1 | - | 20 | 0.9999 |

■ Repeatability Test Results of Tea Leaf Extracts

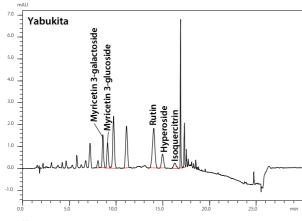
Seven extracts were prepared from one kind of tea (benifuuki) and repeatability test was performed to confirm validity. Table 4 shows the results.

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 |
| Myricetin 3-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.



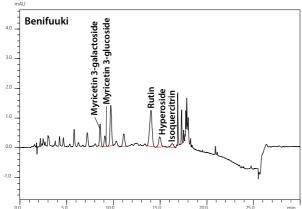


Fig. 3 Chromatograms of Tea Leaf Extracts

Table 5 Flavonol Content in Tea Leaves

| Compound | Content (mg/g) | | | |
|-------------------------|----------------|-----------|--|--|
| Compound | 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 | | |
| Myricetin 3-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.
- 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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