

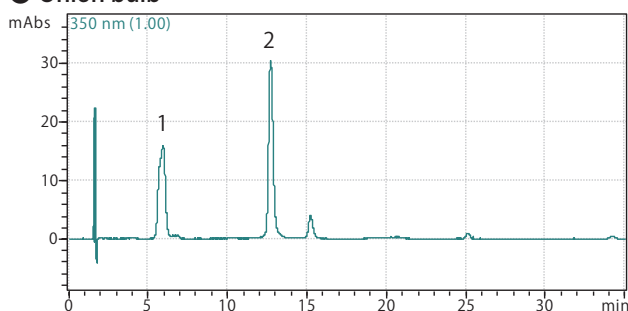
In this note, flavonoids from vegetables were extracted and analyzed using a C<sub>18</sub> column with UV and MS detection.

#### About flavonoids

Flavonoids are a type of polyphenol. The phenol hydroxy group has antioxidant effects, and these compounds are said to be able to prevent some lifestyle diseases.<sup>1</sup> Tea catechins, onion quercetins,<sup>2</sup> soy isoflavones, and hesperidin from citrus fruits<sup>3</sup> are well-known examples; flavonoids are also found in plants such as buckwheat, other Fabaceae (bean family) plants, spinach,<sup>4,5</sup> Apiaceae family plants including parsley,<sup>6</sup> and Cruciferae family vegetables. With some exceptions, such as tea catechins, flavonoids mostly exist as glycosides.

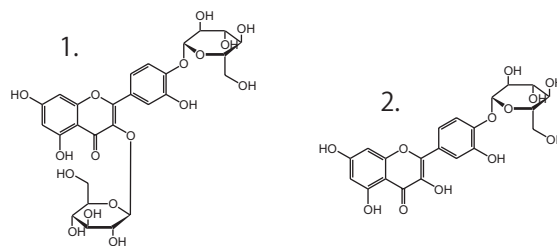
#### Analysis of vegetable flavonoids with HPLC-UV

##### Onion bulb

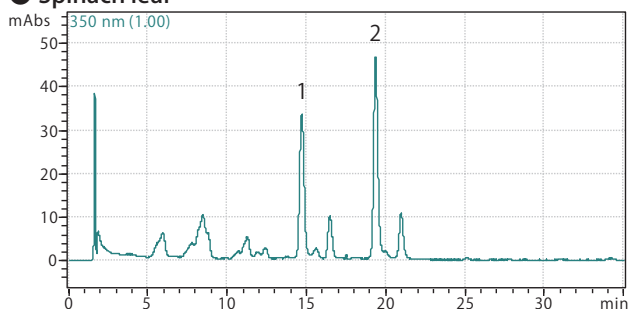


Sample : Onion bulb extract

Peaks : 1. Quercetin diglucoside  
2. Quercetin monoglucoside

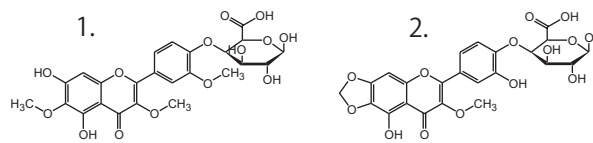


##### Spinach leaf

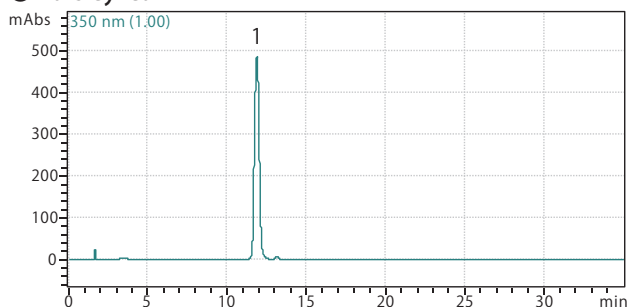


Sample : Spinach leaf extract

Peaks : 1. Spinatocide monoglucuronide  
2. 5,3',4'-trihydroxy-3-methoxy-6:7-methylenedioxyflavone monoglucuronide

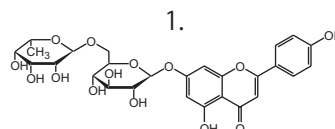


##### Parsley leaf



Sample : Parsley leaf extract

Peaks : 1. Apigenin rutinoside



#### Analysis conditions

Column : COSMOSIL 2.5C<sub>18</sub>-MS-II, 2.0 mmI.D. x 150 mm

Mobile phase : A: 0.1% formic acid (MeOH:H<sub>2</sub>O = 10:90)

B: 0.1% formic acid (MeOH:H<sub>2</sub>O = 90:10)

B conc.

30% → 60% (0 min. → 25 min.),

60% (25 min. → 30 min.),

60% → 30% (30 min. → 30.1 min.),

30% (30.1 min. → 35 min.)

Flow rate : 0.2 mL/min

Temp. : 40°C

Detector : UV 350 nm

Injection vol. : 5 µL

Peaks were identified using LC-MS data and literature values.<sup>2,4,5,6</sup>

## Sample extraction

### (1) Vegetable preparation

#### • Onion bulb

Peel the brown skin off of the onion bulb, and chop the white edible portion finely with a knife.

#### • Spinach leaf

Chop the spinach leaf finely with a knife.

#### • Parsley leaf

Tear the parsley leaf from the stem and chop the leaf finely with a knife.

### (2) Flavonoid extraction

① Mix the chopped vegetable well and measure 10 g for the sample.

② Add 80 mL of extraction solvent (MeOH:H<sub>2</sub>O = 80:20) and shake.

③ Occasionally shake and stir with a glass rod, and leave overnight at room temperature.

④ Filter using a Whatman grade 2 filter paper, and reserve the filtrate.

⑤ Wash the residue by adding extraction solvent.

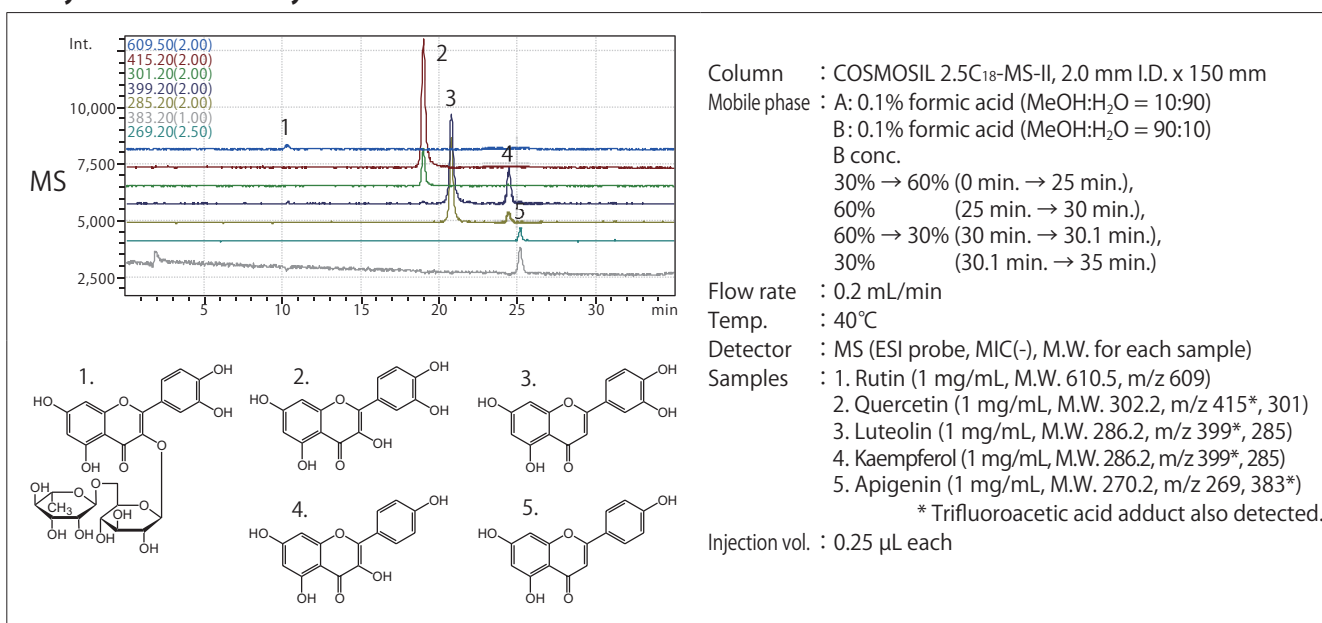
⑥ Filter using a Whatman grade 2 filter paper, and mix the filtrate with that from step ④.

⑦ Add extraction solvent to the liquid from step ⑥ to a total of 100 mL.

⑧ Filter a portion using a Cosmonice Filter W, and use that as the HPLC sample.

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## Analysis of standards by HPLC-MS



## References

1. Inoue, J., Sato, R. KAGAKU TO SEIBUTSU. 2016, vol.54, no. 6, p. 416-420.
2. Okamoto, D., et al. The Horticulture Journal. 2006, vol. 75, no. 1, p. 100-108.
3. Nogata, Y. Bulletin of the National Agricultural Research Center for Western Region. 2005, vol. 5, p. 19-84.
4. Watanabe, M., Ayugase, J. J. Sci. Food Agr. 2015, vol. 95, no. 10, p. 2095-2104.
5. Watanabe, M., Ayugase, J. Food Science and Technology Research. 2015, vol. 62, no. 10, p. 501-507.
6. Plazonic, A., et al. Molecules. 2009, vol. 14, no. 7, p. 2466-2490.

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