Analysis of Functional Ingredients (Anti-Obesity Agents) in Beverages and Foods

Introduction

Previously in Japan, foods that were allowed to claim their functionalities were limited to the “Food for Specified Health Uses (FOSHU)”, individual food that are officially approved by the Japanese Ministry of Health, and the “Functional Nutritional Food”, foods that met the national standard. However, in April 2015, the nation started the “Foods with Functional Claims (FFC)” system to help general consumers obtain correct information and wisely select foods. A FFC product package clearly shows that it is a FFC product and describes its claimed functionalities. FFC can be obtained by submitting the necessary documents to the consumer affairs agency. The submission does not require any national examination to prove ingredient’s safety nor functionality. FFC is approved, as long as the producer states the functionality of the food based on scientific evidences and be responsible to the statement. In March 2021, there are more than 3,500 submitted FFC. It can be said that the FFC market is keep expanding.

In this application, we optimized analytical conditions for the analyses of functional food ingredients – especially focused on anti-obesity agents that are registered as functional food ingredients in the database and which are used in commercial beverages and foods.

Experimental and Results

1. Analysis of acetic acid in vinegar drinks

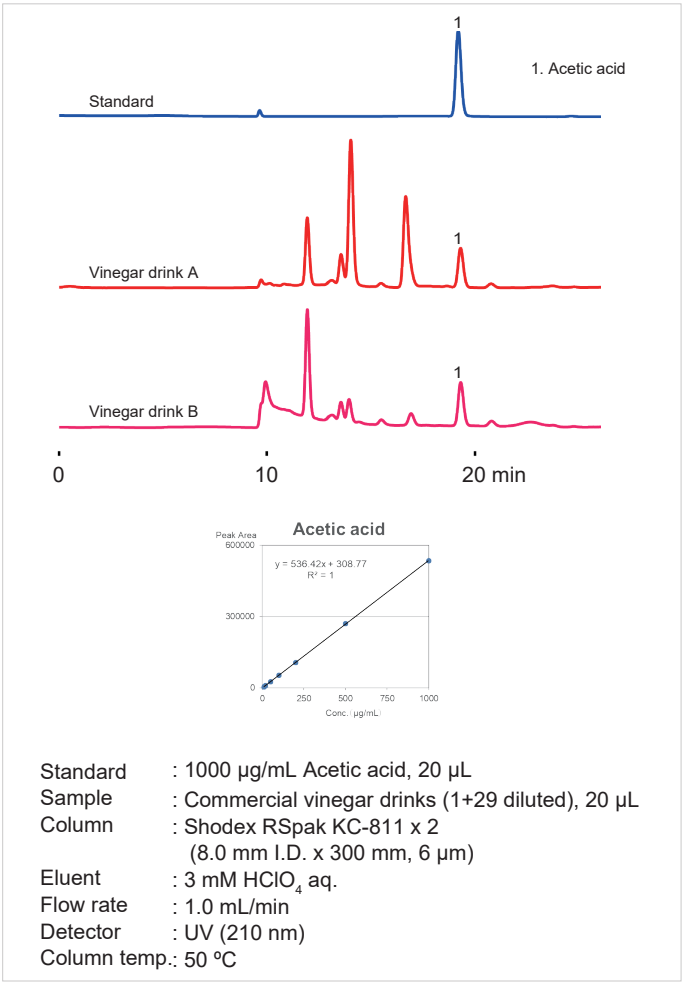
Acetic acid has body fat and triglyceride suppressing effects, and thus expected to work as an anti-obesity agent. Here, we analyzed acetic acid in two commercial vinegar drinks.

Commercial vinegar drinks were diluted with ultra-pure water (1+29), filtered with a membrane filter (0.45 µm), and then the filtrate was used for analysis. A polymer-based ion-exclusion chromatography column, Shodex RSpak KC-811 is generally used to analyze organic acids. We used two KC-811 columns in series for this analysis.

The chromatograms of commercial vinegar drinks show presence of multiple components. Use of two KC-811 columns demonstrated the ability to separate the acetic acid from other components (Fig. 1).

A simple UV detection was used for the analysis; as small amount of impurities in the sample did not require the use of often-used post-column method.

A good linearity was obtained for the calibration curve in the concentration range 1 to 1,000 µg/mL. The recovery rate of acetic acid in vinegar drinks A and B calculated were over 90 %, compared to the information indicated by the manufacturers. Therefore, the developed method demonstrated its suitability to quantify acetic acid in vinegar drinks.



Standard	: 1000 µg/mL Acetic acid, 20 µL
Sample	: Commercial vinegar drinks (1+29 diluted), 20 µL
Column	: Shodex RSpak KC-811 x 2 (8.0 mm I.D. x 300 mm, 6 µm)
Eluent	: 3 mM $HClO_4$ aq.
Flow rate	: 1.0 mL/min
Detector	: UV (210 nm)
Column temp.	: 50 ºC

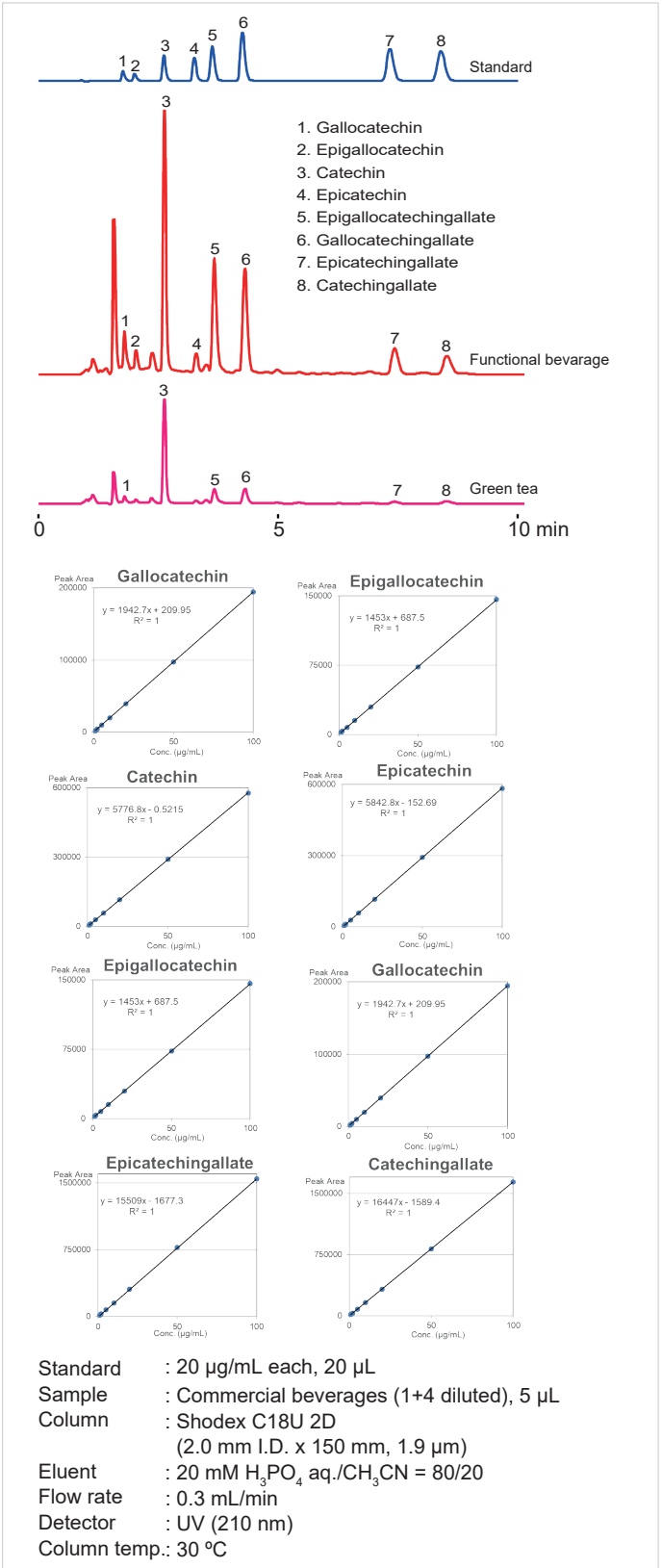
Fig. 1. Chromatograms of acetic acid in standard and commercial vinegar drinks

2. Analysis of catechins in green teas

Catechines have body fat suppressing effects. Here, we analyzed catechins in two commercial green teas and compared their catechin contents.

Commercial green teas were diluted with ultra-pure water (1+4), filtered with a membrane filter (0.45 µm), and then the filtrate was used for analysis. We used a Shodex C18U 2D, an ODS column packed with organic/inorganic silica hybrid particles which provides excellent resolution, mechanical stability, and improved alkali durability.

A rapid analysis was performed for the analysis of eight catechins listed in the FFC data base. C18U 2D completed the analysis of eight catechins within ten minutes (Fig. 2).



Standard : 20 μg/mL each, 20 μL
Sample : Commercial beverages (1+4 diluted), 5 μL
Column : Shodex C18U 2D (2.0 mm I.D. x 150 mm, 1.9 μm)
Eluent : 20 mM H_3PO_4 aq./CH_3CN = 80/20
Flow rate : 0.3 mL/min
Detector : UV (210 nm)
Column temp.: 30 ºC

Fig. 2. Chromatograms of catechins in standard and commercial green teas

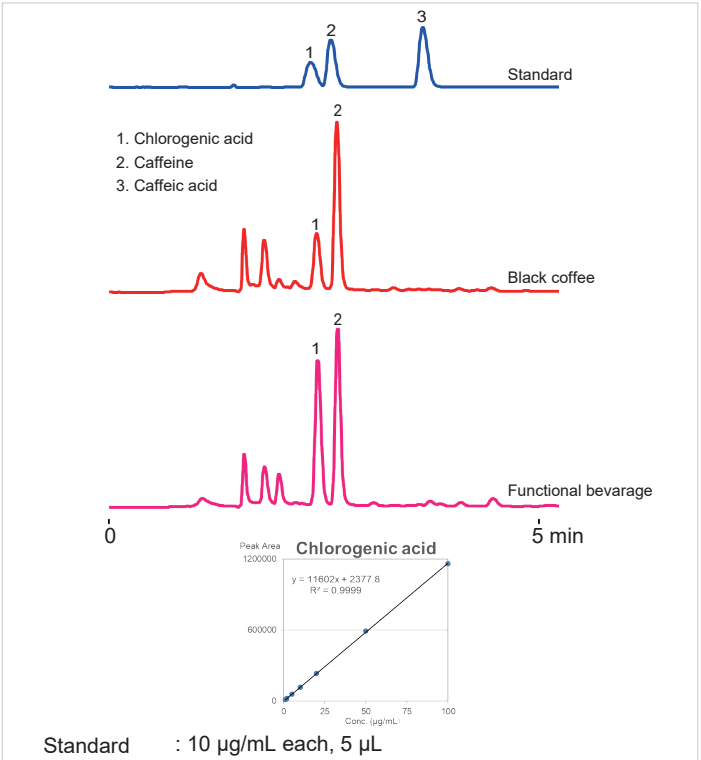
Good linearities were obtained for the calibration curves in the concentration range 1 to 100 μg/mL. The green tea sold as a FFC contained more tea catechins than the regular green tea. The recovery rate of total catechin in the green tea sold as a FFC was over 90 %, compared to the information indicated by the manufacturer. Therefore, the developed method demonstrated its suitability to quantify catechins in green tea.

3. Analysis of chlorogenic acid in coffee

Chlorogenic acid has blood glucose and body fat suppressing effects. Here, we analyzed chlorogenic acid in two commercial no-sugar coffee drinks.

Commercial no-sugar coffee was diluted with ultra-pure water (1+4), filtered with a membrane filter (0.45 μm), and then the filtrate was used for analysis. We used a Shodex C18U 2D for the analysis as we did for “2. Analysis of catechins in green teas”.

The analysis of chlorogenic acid and two main components in coffee, caffeine and cafeic acid, was completed within five minutes (Fig. 3). This rapid analysis also showed the presence of other impurities in coffee eluted within 10 minutes. A good linearity was obtained for the calibration curve in the concentration range 1 to 100 μg/mL.



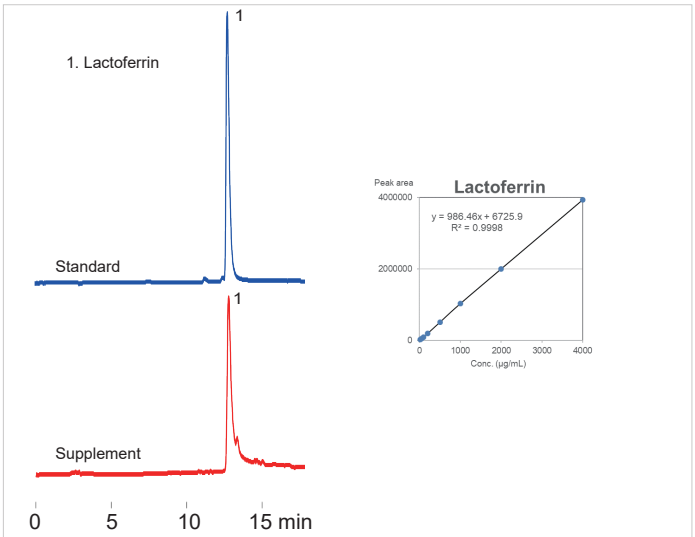
Standard : 10 μg/mL each, 5 μL
Sample : Commercial beverages (1+4 diluted), 20 μL
Column : Shodex C18U 2D (2.0 mm I.D. x 150 mm, 1.9 μm)
Eluent : 20 mM H_3PO_4 aq./CH_3CN = 80/20
Flow rate : 0.3 mL/min
Detector : UV (210 nm)
Column temp.: 30 ºC

Fig. 3. Chromatograms of chlorogenic acid in standard and commercial coffee

4. Analysis of lactoferrin in a supplement

Lactoferrin has visceral fat reducing effects and body fat suppressing effects. It also helps to improve the gastrointestinal environment by suppressing the growth of "bad" bacteria and increasing the "good" bacteria. Here, we analyzed lactoferrin in a commercial supplement tablet claims to contain lactoferrin.

A commercial supplement tablet claims to contain lactoferrin was ground using a mortar and pestle to make it into powders. We transferred 200 mg of the powder in a beaker, added 50-mL ultrapure water, and ultrasonicated for 10 minutes. The supernatant was filtered with a No. 5B filter paper and further filtered with a membrane filter (0.45 µm). The obtained filtrate was used for analysis. We used a Shodex Asahipak C4P-50 4D, a polymer-based reversed-phase column modified with butyl functional group. The column is applicable for the lactoferrin concentrate analysis method in "Japan's specifications and standards for food additives, 9th edition". We employed their method with slight modification. Presence of a few impurities found in the supplement allowed a simple analytical condition (Fig. 4).



Standard : 4000 µg/mL Lactoferrin, 25 µL
Sample : Supplement extract, 25 µL
Column : Shodex Asahipak C4P-50 4D (4.6 mm I.D. x 150 mm, 5 µm)
Eluent : (A) H_2O/TFA = 100/0.05 (v/v)
(B) CH_3CN/TFA = 100/0.05 (v/v)
Step gradient → 0 - 50 B % (0 - 10 min)
→ 50 B % (10.01 - 15 min)
→ 50 - 0 B % (15 - 15.01 min)
→ 0 B % (15.01 - 20 min)
Flow rate : 0.8 mL/min
Detector : UV (280 nm)
Column temp. : 40 °C

Fig. 4. Chromatograms of lactoferrin in standard and commercial supplement tablet

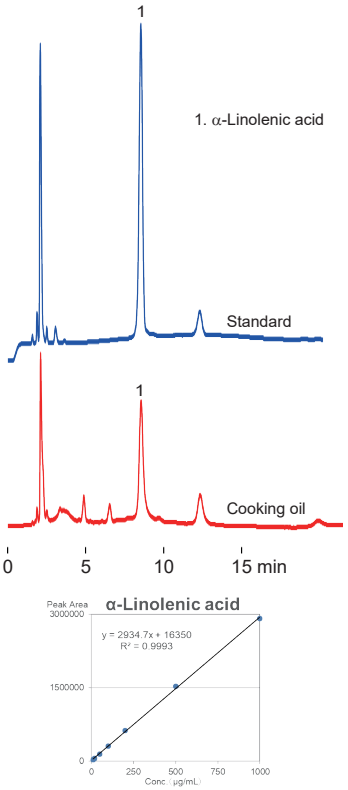
A good linearity was obtained for the calibration curve in the concentration range 10 to 4,000 ug/mL. The recovery rate of lactoferrin was over 90 %, compared to the information indicated by the manufacturer. Therefore, the developed method demonstrated its suitability to quantify lactoferrin in a supplement tablet.

5. Analysis of α-linolenic acid in a cooking oil

α-Linolenic acid has body fat reducing effects, and also helps to improve cholesterol level and supports a healthy blood pressure. Here, we analyzed α-linolenic acid in a commercial cooking oil.

A commercial cooking oil was diluted (1+1) with tetrahydrofuran (THF), filtered with a membrane filter (0.45 µm), and then the filtrate was used for analysis. We used a Shodex Asahipak ODP-50 4D, a polymer-based reversed-phase column modified with octadecyl functional group.

A good linearity was obtained for the calibration curve in the concentration range 1 to 1,000 µg/mL (Fig. 5). The recovery rate of α-linolenic acid was over 90 %, compared to the information indicated by the manufacturer. Therefore, the developed method demonstrated a suitability to quantify α-linolenic acid in cooking oil. Note that dibutylhydroxytoluene (BHT) is often added to THF to prevent the oxidation of THF. However, BHT was found to elute at the same time as α-linolenic acid in this chromatographic condition, therefore we used THF without BHT to dissolve the sample.



Standard : 1000 μg/mL α-Linolenic acid in THF, 5 μL
Sample : Cooking oil (1+1 diluted), 5 μL
Column : Shodex Asahipak ODP-50 4D (4.6 mm I.D. x 150 mm, 5 μm)
Eluent : 0.1 % H_3PO_4 in (H_2O/CH_3CN = 35/65)
Flow rate : 1.0 mL/min
Detector : UV (215 nm)
Column temp.: 40 ºC

Fig. 5. Chromatograms of α-linolenic acid in standard and commercial cooking oil

Conclusions

This technical article demonstrated the Shodex columns' feasibility for the analysis of various functional ingredients in beverages and foods. They were detected without the influence of other components present in the supplements.

In Japan, when applying to resister a food/food product as a FFC, which analytical method to analyze the target functional ingredients is up to the producer/claimer, as long as the method provides scientifically reliable result. Therefore, the methods introduced in this technical article using the Shodex columns may also be applicable for the analysis of those functional ingredients.

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TA. NO. 018 (1) 21. D. MAY. P