

Analysis of Functional Components Procyanidins in Apple Juice

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User Benefits

- ◆ Procyanidins in apple juice were analyzed by the method described in Japanese Agricultural Standard (JAS 0024) .
- ◆ Determination of procyanidins in apple juice can be performed.

Introduction

Antioxidants are considered to inhibit the generation and function of active oxygen, which interfere with human immune system. Polyphenols are often paid attention as antioxidants. Procyanidins are one type of polyphenols and are said to account for approximately 60% of the polyphenols in apples. It has been reported to have various biological regulatory functions such as anti-allergy, anti-tumor, regulation of sugar and lipid metabolism, hair growth, prevention of arteriosclerosis, and anti-aging. In addition to apples, it is abundant in cacao, black soybeans, cinnamon, and nuts. In this study, we analyzed procyanidins in apple juice.

Analysis of standard solution

Procyanidins have polymerized structures based on epicatechin and catechin, known to range from 2 to 15 mers. In general, however, low molecular weight compounds make up the majority of procyanidins. Figure 1 shows the structural formulae of catechin, epicatechin, procyanidin B2, and procyanidin C1.

The standard method for the determination of procyanidins in apple juice (JAS 0024) ¹⁾ was established by the Ministry of Agriculture, Forestry and Fisheries on March 31, 2022. This application was performed following the procedures in JAS 0024 ¹⁾. Analytical conditions are shown in Tables 1 and 2, and chromatogram of standard solution is shown in Figure 2. The standard solution was prepared from a mixture of acetone/acetic acid/water containing L(+)-ascorbic acid (acetone/acetic acid/water = 140:1:59 (volume ratio) solution with 0.5 g of L(+)-ascorbic acid per 1 L). The calibration curve is shown in Figure 3. Using procyanidin B2 as a standard solution, the peak area of the dimer or higher is determined and compared to that of procyanidin B2 for determination.

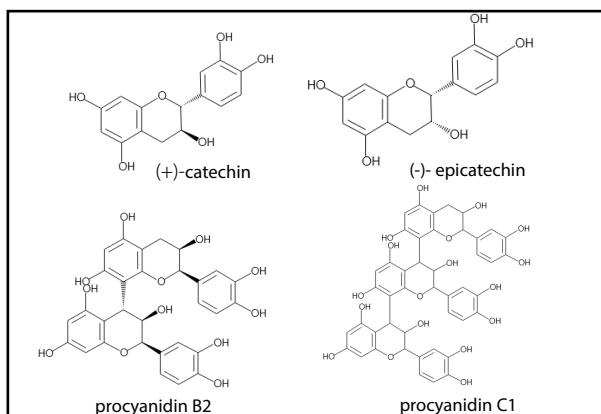


Fig.1 Structure of catechin, epicatechin and procyanidins

Table 1 Analytical conditions

Column	: GL Science Inc. Inertsil WP300 Diol 250 mm × 4.6 mm I.D., 5 μm
Mode	: Low pressure gradient (Mixer volume : 0.5 mL)
Mobile phase	: A) Acetonitrile/Acetic Acid = 98:2 B) Methanol/Water/Acetic Acid = 95:3:2
Flow rate	: 1.0 mL/min
Column temp.	: 35 °C
Injection volume	: 5 μL
Vial	: SHIMADZU Labtotal for LC 1.5mL, Glass *1
Sample cooler	: 4 °C
Detection	: Fluorescence detector(Cell temp. : 40 °C) : Ex. 230 nm, Em. 321 nm

*1 : P/N 227-34001-01

Table 2 Time Program

Time(min)	A conc	B conc.
0.00	93	7
1.50	93	7
1.51	2	98
10.00	2	98
10.01	93	7
20.00	93	7

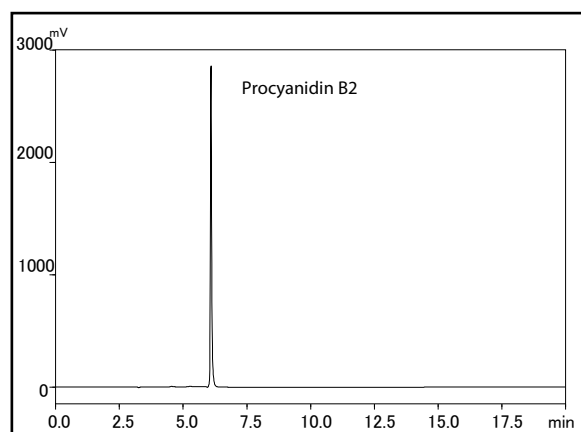


Fig.2 Chromatogram of 40 μg/mL Procyanidin B2

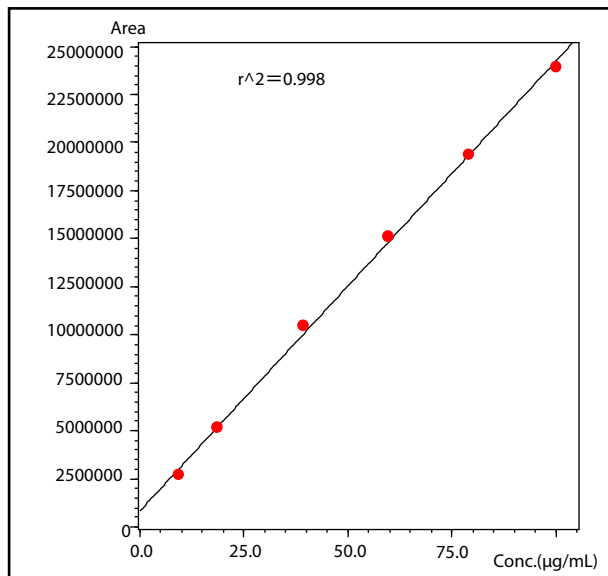


Fig. 3 calibration curve of Procyanidin B2

■ Analysis of apple juice

2 mL of apple juice (not from-concentrate juice) was taken in a 10 mL volumetric flask, 0.1 mL of 50 g/L ascorbic acid solution, 0.05 mL of acetic acid, and 7 mL of acetone were added. Then water was added to make 10 mL. After well-shaking, the mixture was filtered prior to analysis. Obtained chromatograms of for the two types of apple juice are shown in Figures 4 and 5.

Two peaks appeared at the elution position of procyanidin B2 in both chromatograms. This is due to the elution of dimer or higher procyanidins. These peaks are collectively determined as procyanidins.

■ Conclusion

We determined procyanidins in apple juice in accordance with the Japanese Agricultural Standard (JAS 0024)¹⁾.

This analysis was obtained through participation in an inter-laboratory joint test conducted for the establishment of JAS 0024¹⁾. We would like to thank the Food and Agricultural Materials Inspection Center for lending us the columns for the joint test. We would like to express our appreciation.

<Reference>

1) Japanese Agricultural Standard JAS 0024 : determination of the procyanidins in apple juice-High performance liquid chromatographic method

<Related Patent>

JP.6508741B2

<YouTube URL>

[JAS0024 Determination of the procyanidins in apple juice \[FAMIC\] - YouTube](#)

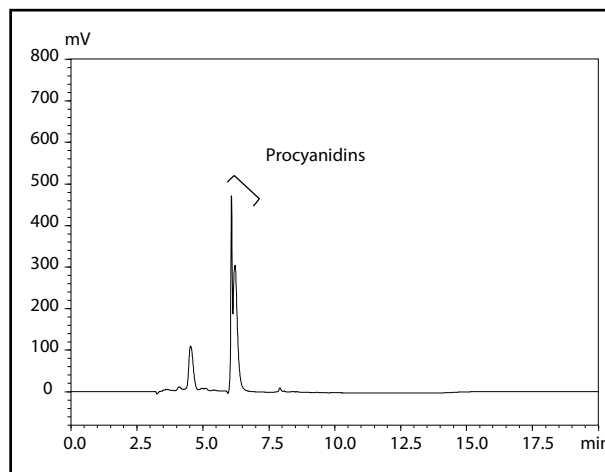


Fig.4 Chromatogram of apple juice A (not from-concentrate juice)

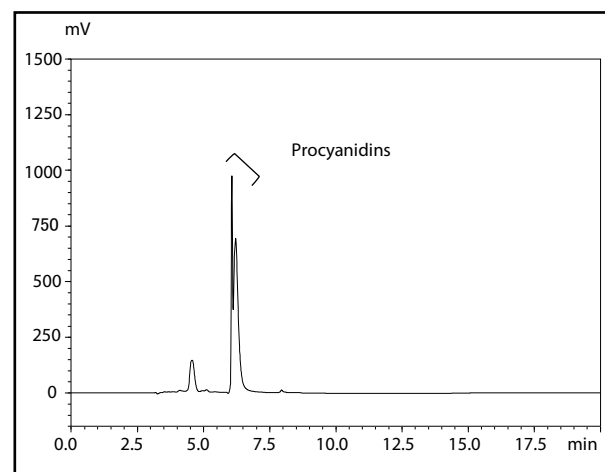


Fig.5 Chromatogram of apple juice B (not from-concentrate juice)

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