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Functional Food Analysis / i-series LC-2050C

Application News

Determination of Functional Component in Agricultural Product: β-cryptoxanthin in Mandarin Orange by HPLC Method

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User Benefits

- Quantitative determination of functional component of β-cryptoxanthin (BCR) in fresh mandarin orange samples using an i-series LC-2050C with a high sensitivity UV detector and an excellent ODS column for separation.
- High throughput, accurate and reliable method to measure the functional nutrient in fresh fruits.

■ Introduction

Satsuma Mandarin is listed as a functional food in Japan, which can be recognized by Functional Food Claims (FFC), a new regulatory system of health claim introduced in Japan in 2015. The Japanese Agricultural Standards (JAS) are established to support the FFC new functional food system [1]. β-cryptoxanthin can be found in fruits such as mandarin orange, papaya, and mango. β-cryptoxanthin has several beneficial effects on human health, such as acting as an antioxidant on scavenging free radicals from our body for preventing cancer, being a precursor to vitamin A on improving human health vision, reducing the signs of ageing, etc. In this application news, a HPLC method associated with the JAS 0003 reference method [2] is described for quantitation of BCR in fresh mandarin orange. The sample preparation and HPLC analysis were performed in accordance with the JAS method 0003.

■ Experimental

Reagents and standard

β-cryptoxanthin standard, reagents, chemicals, and apparatus used in sample preparation were prepared in reference to JAS 0003 monograph [2]. The high purity standard BCR was purchased from a local chemical supplier. Reagents and chemicals such as pyrogallol, ascorbyl palmitate, potassium hydroxide (KOH), sodium chloride and sodium sulfate are analytical grade. Solvents including n-hexane, ethyl acetate, petroleum ether, ethanol, methanol, chloroform and 2-propanol are HPLC grade.

HPLC analytical conditions

The analysis was carried out using Shimadzu i-series LC-2050C system. LabSolutions workstation was used for data acquisition and data analysis. The system configuration, HPLC column and detailed parameters are compiled into Table 1.

Table 1. Analytical conditions of β-cryptoxanthin by HPLC

Column	Shim-pack™ GIS-HP C18, 150 × 3.0 mm, 3 µm
Flow rate	1.0 mL/min
Mobile phase	methanol-chloroform mixture (96/4, v/v) with 0.12 mM ascorbyl palmitate
Elution mode	Isocratic elution
Oven Temp.	40°C
Detection	455 nm
Injection volume	10 µL

■ Results and Discussion

Sample preparation

Fresh mandarin orange samples were obtained from the local market for this testing. The samples were stored under cold conditions kept at 5°C. Prepare all mandarin samples in 1 day to avoid the change in BCR concentration. Perform steps 2 to 5 on each sample first. One whole mandarin is for 1 analysis.

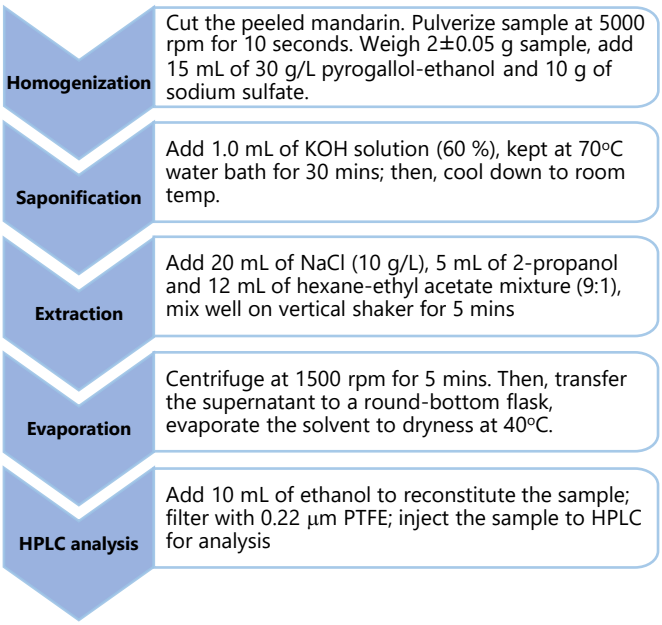

Figure 1. Sample preparation procedure for BCR analysis in mandarin orange by HPLC (JAS 0003 monograph).

The JAS 0003 monograph describes in details the procedure of sample preparation as outlined in Figure 1, which includes four steps before the sample was injected to LC-2050C for analysis.

Calibration curve

Figure 2 shows the HPLC result of the BCR standard (level L1). The retention time of BCR was 3.31 min. A linear calibration curve was set up using a calibration series from L1 to L4 (0.25, 0.5, 1 and 2 µg/mL) of BCR dissolved in ethanol from a BCR stock solution of 10 µg/mL. The concentration of BCR of the stock solution prepared or received must be measured by UV-VIS absorbance measurement to obtain the accurate value (see section 4.23.2 [2]). After this correction, the actual concentrations of L1~L4 calibration series obtained were 0.247~1.98 µg/mL. The correlation coefficient of the linear calibration curve for the above range was 0.999, which met the requirement (r > 0.995) stated in JAS 0003.

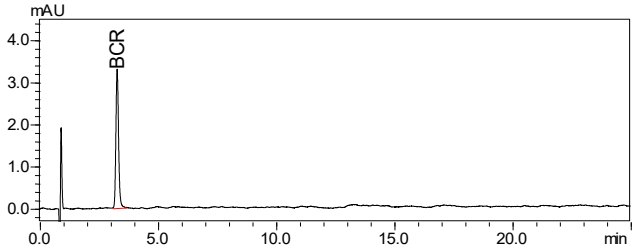

Figure 2. Chromatogram of BCR standard of 0.25 µg /mL in neat solvent.

Quantitation of β-cryptoxanthin in mandarin orange

The contents of BCR (mg/kg) in the mandarin orange samples were determined by the established HPLC method. Two mandarin samples were prepared and measured. The results of BCR content in mandarin can be calculated from the concentrations of BCR measured using a formula as shown below. The average results of BCR in the mandarin orange samples after calculation was ~ 12.3 mg/kg, which fell in the BCR mean content range shown in the reference data (see Table A.1, in the Annex A).

$$W(\mathrm{mg/kg}) = \frac{C \times V \times d1}{M \times d2}$$

Where

W = Amount of BCR in mandarin samples (mg/kg)
C = Concentration of BCR in the samples (µg/mL)
V = Volume of volumetric flask used (5 mL)
M = Weight of testing samples (g)
d1 = Constant volume at time of extraction (50 mL)
d2 = Saponification fraction volume (10 mL)

Identification

Identification of BCR peak in extract samples (Figure 3) relies on matching the retention time (RT) with BCR standard. The RTs of BCR in the samples matched perfectly (shift less than 0.1 min) with standard without obvious interference.

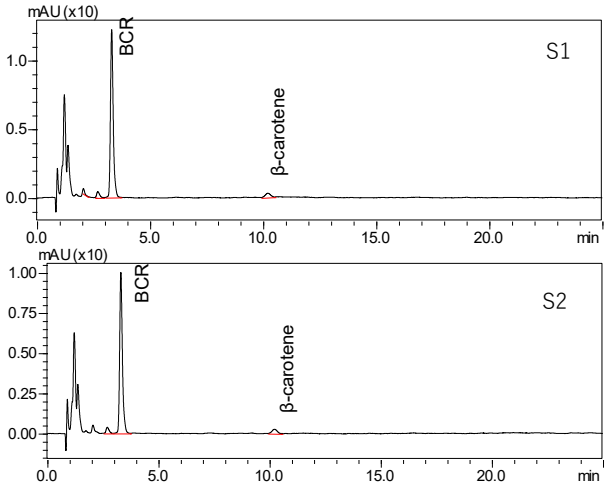

Figure 3. Representative chromatogram profiles of BCR mandarin extracts, S1 (top) and S2 (bottom).

Precision

Interlaboratory tests are required essentially to verify the precision of analysis results. Details of the interlaboratory tests are described in Annex A in JAS 0003 monograph. Repeatability limit ($r = 2.8 \times S_r$) and reproducibility limit ($R = 2.8 \times S_R$) were used to evaluate the precision of the analyses in laboratories intended to provide testing of BCR in mandarin orange following JAS method. The procedure shown in this application news was considered as a practice of interlaboratory tests. The results (data not shown) were compared with that obtained by an accredited laboratory to verify the precision.

■ Conclusion

This work demonstrates the procedure and quantitation results of β-cryptoxanthin in fresh mandarin orange analyzed by a HPLC method associated with JAS0003 monograph. The process from sample preparation to HPLC analysis was performed as a practice for interlaboratory tests as described in Annex A of JAS0003.

■ References

1. Development of functional agricultural products and use of a new health claim system in Japan, Trends in Food Science & Technology (69) 2017, 324-332.
2. Japanese Agricultural Standards Method JAS 0003: 2019

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