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Functional Food Analysis / HPLC

Application News

Determination of Functional Component in Agricultural Product: Lutein in Fresh Spinach by HPLC Method

Zhe Sun, Zhaoqi Zhan

User Benefits

- Full procedure from sample collection, transportation, pretreatment to quantitative analysis of lutein in spinach by HPLC method following the JAS 0008 method
- Method evaluation of quantitation, identification and precision by interlaboratory tests

■ Introduction

In 1990s, functional foods labelled as "Food for Specified Health Uses" or FOSHU products was introduced in Japan domestic market [1]. Functional foods contain ingredients that offer specific health benefits. For example, lutein ($C_{40}H_{56}O_2$), a carotenoid compound found in spinach and other green leaf vegetables, has several beneficial effects on eye health. Hence, Spinach (*Spinacia oleracea L.*) is listed as the Food with Functional 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. In this application news, a HPLC method associated with the JAS 0008 (2019) monograph [2] is described and applied for the quantitation of lutein in fresh spinach. The analysis results of the testing samples are compared with that obtained from an accredited laboratory (data not shown) to confirm the precision.

■ Experimental

Reagents and standard:

Reagents, lutein standard and apparatus used in sample preparation were prepared in reference to JAS 0008 monograph [2]. High purity lutein standard was purchased from Sigma Aldrich (07168-1MG). Reagents such as pyrogallol, BHT (butylated hydroxytoluene), potassium hydroxide (KOH), sodium chloride, ammonium acetate are analytical grade. Solvents including n-hexane, ethyl acetate, ethanol, methanol and acetonitrile are HPLC grade.

HPLC analytical conditions:

A Prominence HPLC was used in this analysis, which consists of a binary pump system LC-20AD, autosampler SIL-20AC, column oven CTO-20A and a UV-VIS detector SPD-20AV. LabSolutions workstation was used for data acquisition and data analysis. The HPLC column and detailed parameters are compiled into Table 1.

Table 1. Analytical conditions of lutein by HPLC

Column	YMC carotenoid, 150 × 4.6 mm, 3 µm
Flow rate	1.0 mL/min
Mobile phase	A : ACN-MeOH (75/20 v/v, 15 mM ammonium acetate) B : Ethanol
Elution mode	Gradient elution program: 0.01-13 min (5 %B) ➔ 13.01 min (95 %B) ➔ 20 min (95 %B) ➔ 20.01 min (5 %B) ➔ 25 min
Oven Temp.	40°C
Detection	445 nm
Injection volume	10 µL

■ Results and Discussion

Sample preparation:

Fresh spinach samples were collected in Japan for this testing. The samples were shipped to this laboratory by air under cold conditions kept at 5°C. Weight losses of the samples after 7 days from harvest and packing were measured. They were within 3~6%, except one sample showing 11% weight loss.

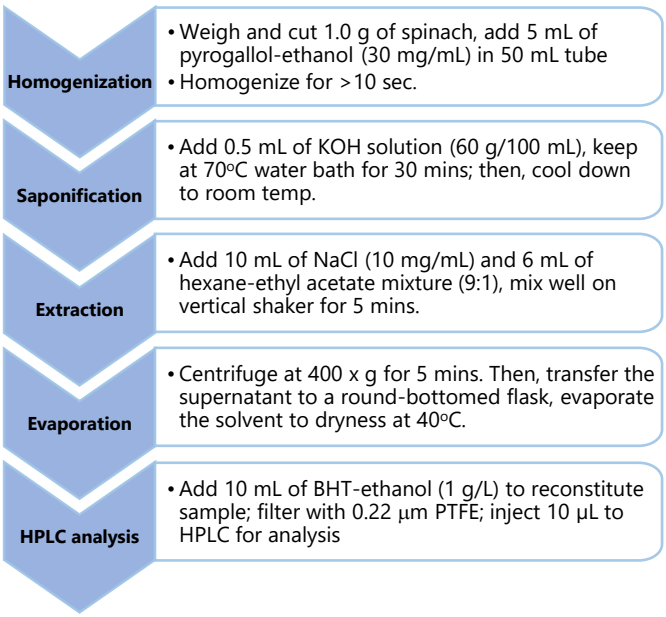

Figure 1. Sample preparation procedure for lutein analysis in spinach by HPLC (JAS 0008 monograph).

The JAS 0008 monograph describes in details the procedure of sample preparation as outlined in Figure 1, which includes four steps before the sample is injected to HPLC for analysis.

Calibration curve:

Figure 2 shows the HPLC result of a standard (L1). The retention time of lutein was 7.8 mins. A linear calibration curve was set up using a calibration series from L1 to L5 (1, 2, 5, 10 and 20 µg/mL) of lutein in BHT-ethanol solution (1g/L) from a lutein stock of 100 µg/mL. The concentration of lutein of the stock solution prepared or received must be measured by UV-VIS absorbance measurement to obtain accurate value (see section 4.22.2 [2]). After this correction, the actual concentrations of L1~L5 calibration series were 0.728~14.568 µg/mL. The correlation coefficient of the linear calibration curve for the above range was 0.998, which met the requirement (r > 0.995) stated in JAS 0008. The S/N ratio of L1 (0.728 µg/mL) was greater than 200.

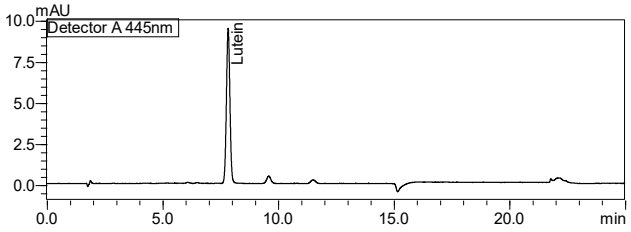

Figure 2. Chromatogram of lutein standard of 0.728 µg /mL (L1) in diluent.

Quantitation of lutein in spinach:

The contents of lutein (mg/kg) in nine spinach samples were determined by the HPLC method. The results are shown in Table 2, calculated from the concentrations of lutein measured using a formula as below [2].

$$W(mg/kg) = \frac{4 \times C \times V}{M}$$

Where

W = Content of lutein in spinach (mg/kg)
C = Concentration of lutein in extract (µg/ml)
V = Constant volume of dissolution (10 mL)
M = Mass of test sample used (g)

Table 2 Contents of lutein in spinach samples by HPLC

Sample	Weight (g)	Conc. (µg/mL)	Cont. (mg/kg)
1a	1.02	1.55	60.9
4a	1.04	1.76	67.5
7a	1.01	1.66	65.6
10a	1.03	1.7	66.1
13a	1.02	1.86	72.8
16a	1.03	1.34	51.9
19a	1.03	1.82	70.6
22a	1.04	1.57	60.5
25a	1.04	1.49	57.3

Identification:

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

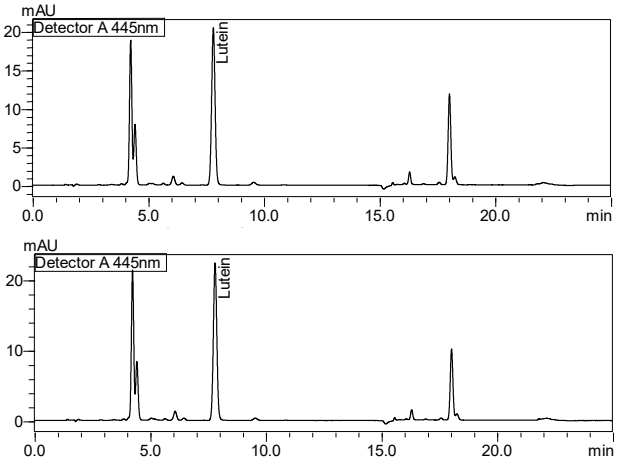

Figure 3. Representative chromatogram profiles of lutein extracts, 1a (top) and 10a (bottom).

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 0008 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 lutein in spinach following JAS. The procedure and results shown in this application news were considered as a practice of interlaboratory tests. The results (Table 2) were compared with that obtained by an accredited laboratory (data not shown) to verify the precision.

■ Conclusion

This work demonstrates the procedure and quantitation results of lutein in fresh spinach analyzed by a HPLC method associated with JAS0008 monograph. The process from sample preparation to HPLC analysis was performed as a practice for interlaboratory tests as described in Annex A of JAS0008.

■ References

1. Shun Iwatani, Naoyuki Yamamoto; Food Science and human wellness 8 (2019) 96-101.
2. Japanese Agricultural Standards Method JAS 0008: 2019

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