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Application News

No. L582

High Performance Liquid Chromatography

Quantitative Analysis of Carotenoids in Tea Leaves

In collaboration with the National Agriculture and Food Research Organization, Shimadzu Corporation has been developing a simple, quick and accurate method of analyzing functional components in agricultural and food products.

This report introduces a quantitative method for carotenoids analysis in tea leaves and presents the results obtained in two kinds of them. Carotenoids are classified as aliphatic hydrocarbons containing 40 carbon atoms. So far, more than 700 types of carotenoids have been identified in nature. For examples, lycopene presents in tomatoes and lutein in spinach. Because all these compounds have antioxidant properties (in common with polyphenols), carotenoids draw attention as promising functional components in human diet. In this report, the carotenoids shown in Table 1 were analyzed.

M. Kawashima

Table 1 Target Compounds

Compound
Astaxanthin
α-carotene
β-carotene
β-cryptoxanthin
Zeaxanthin
Neoxanthin
Lutein

Sample Pretreatment

The extraction was performed following the conditions determined in the pre-existing method for lutein analysis by Japanese Agricultural Standards (JAS)[1), 2), 3), 4)]. The workflow is shown in Fig. 1. However, with this pretreatment, astaxanthin is decomposed during the saponification process. So, it is not possible to quantify astaxanthin in tea leaves. To quantify astaxanthin, enzymatic hydrolysis is required.

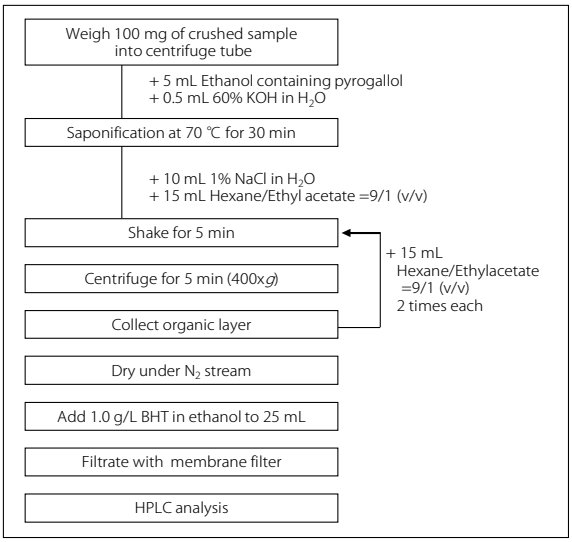

Fig. 1 Pretreatment Workflow

Analytical Conditions

The analytical conditions were determined in the reference of the method for lutein analysis by JAS[1)]. The analytical conditions are shown in Table 2.

Table 2 Analytical Conditions

System	:	Nexera™ X3
Column	:	YMC Carotenoid (150 mm × 4.6 mm I.D., 3 µm)
Mobile phases	:	A) 5 g/L Ammonium acetate in Methanol /Acetonitrile=4/15 (v/v) B) Ethanol
Gradient Program	:	B conc. 5% (0-1.00 min) - 50% (20.00 min) -95% (20.01-25.00 min) -5% (25.01-30.00 min)
Flow rate	:	1.0 mL/min
Column Temp.	:	40 °C
Injection volume	:	10 µL
Detection	:	PDA 438 nm (Neoxanthin), 445 nm (α-carotene, Lutein), 450 nm (β-carotene, β-cryptoxanthin, Zeaxanthin), 470 nm (Astaxanthin)

Analysis Results of Standards

The linearities were determined by the standards analysis. Fig. 2 shows the calibration curves and Fig. 3 shows representative chromatograms.

Table 3 shows the dynamic range and the coefficients of determination. Good linearities were obtained with a coefficient of determination (R^2) ≥ 0.999 for all compounds.

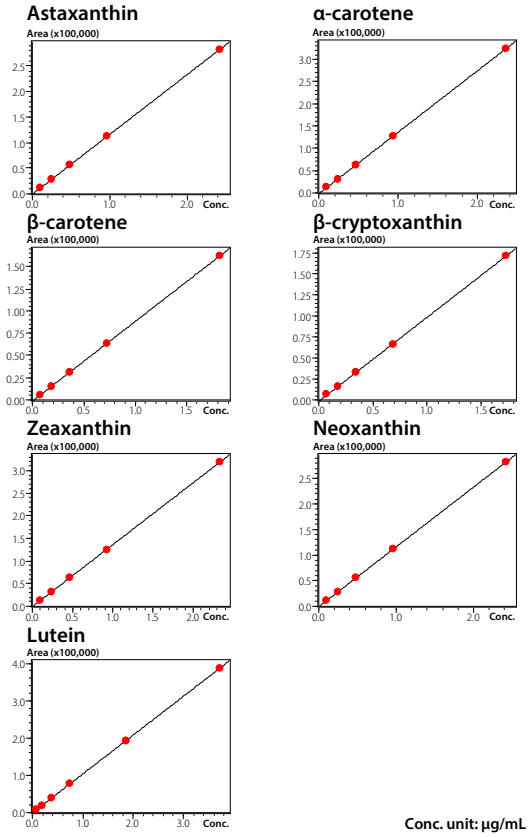

Fig. 2 Calibration Curves

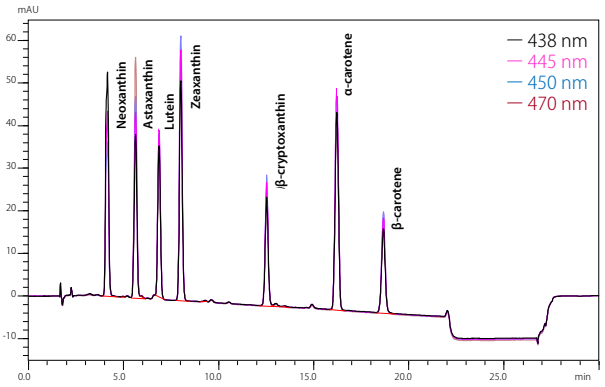

Fig. 3 Chromatograms of Standard Samples

Table 3 Linear range and Coefficient of determination (R^2)

Compound	Linear range (μg/mL)			Coefficient of determination (R^2)
Astaxanthin	0.094	—	2.357	0.9999
α-carotene	0.094	—	2.353	0.9999
β-carotene	0.073	—	1.818	0.9999
β-cryptoxanthin	0.069	—	1.728	0.9997
Zeaxanthin	0.093	—	2.329	0.9999
Neoxanthin	0.097	—	2.417	0.9999
Lutein	0.074	—	3.716	0.9999

Repeatability Test Results of Tea Leaf Extracts

Seven extracts were prepared from one kind of tea (Yabukita) and repeatability test was performed to confirm validity. Table 4 shows the results.

Table 4 Repeatability Test Results (n=7)

Compound	Repeatability (%RSD)
α-carotene	2.87
β-carotene	7.99
β-cryptoxanthin	- (< LLOQ)
Zeaxanthin	6.03
Neoxanthin	9.29
Lutein	4.04

Quantitative Results for Tea Leaves

The extracts of two kinds of tea (Yabukita, Benifuuki) were analyzed to determine the content of carotenoids. Fig. 4 shows the chromatograms and Table 5 shows the calculated content of each carotenoid in tea leaves.

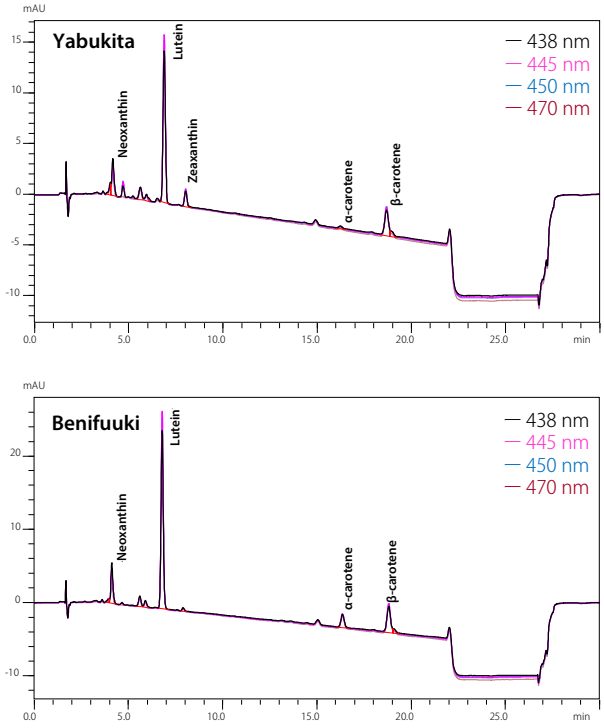

Fig. 4 Chromatograms of Tea Leaf Extracts

Table 5 Carotenoid Content in Tea Leaves

Compound	Content (mg/g)	
	Yabukita	Benifuuki
α-carotene	0.92	4.7
β-carotene	11.3	16.3
β-cryptoxanthin	< LLOQ	< LLOQ
Zeaxanthin	3.19	< LLOQ
Neoxanthin	6.41	9.5
Lutein	34.0	53.7

Conclusion

- Using the Nexera series, simultaneous analysis of carotenoids was performed.
- The carotenoids quantification results show a difference in content depending on the kind of tea leaves.

This analysis method was developed in collaboration with the National Agriculture and Food Research Organization (scheduled from April, 2019 to March, 2022) at the Collaborative Research Laboratory for Analysis of Food Functionality in Shimadzu's Healthcare R&D Center. The analysis method and analysis data presented in this report were provided by Mr. Hironori Juichi and Ms. Yayoi Ichiki, researchers at the National Agriculture and Food Research Organization.

<References>
1) Japanese Agricultural Standard Determination of the lutein in spinach — High-performance liquid chromatographic method (JAS 0008)
2) Standards for Labelling of Food Items (Consumer Affairs Agency Food Labeling Division Notification No. 139 issued by Deputy Director of Consumer Affairs Agency dated March 30, 2015) Appendix Analytical Methods for Nutritional Composition in Foods
3) Analytical Manual for Standard Tables of Food Composition in Japan 2015 (Seventh Revised Edition), Chapter 3 Vitamins (https://www.mext.go.jp/a_menu/syokuhinseibun/1368931.htm)
4) Japanese Agricultural Standard Determination of the β- cryptoxanthin in 'Satsuma Mandarin' — High-performance liquid chromatographic method (JAS 0003)

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