

Nondestructive Analysis of Carotenoids in Fruits and Vegetables
Using Raman Spectroscopy

January 2021

Risa HARA

Nondestructive Analysis of Carotenoids in Fruits and Vegetables
Using Raman Spectroscopy

A Dissertation Submitted to
the Graduate School of Science and Technology,
University of Tsukuba
in Partial Fulfillment of Requirements
for the Degree of Doctor of Philosophy in Agricultural Science

Doctoral Programs in Agricultural Sciences,
Degree Programs in Life and Earth Sciences

Risa HARA

Abstract

Currently, the functional components of fruits and vegetables have attracted consumers, and the requirement for displaying the amount of functional components is increasing. Therefore, in this dissertation, the non-destructive analysis of carotenoids contained in fruits and vegetables by Raman spectroscopy are examined, and suggestions are made for the development of carotenoid sensors for field analysis.

In Chapter 1, the needs for measuring functional components from the market and the characteristics of sensors required in the field of agriculture are considered. Next, the advantages of Raman spectroscopy in the measurement of carotenoids are summarized. Finally, the equipment configuration of the Raman spectrometer is described, and how the Raman spectrometer achieves miniaturization and cost reduction is explained. Based on the results, the challenges for the development of a carotenoid sensor that non-destructively analyzes carotenoids in fruits and vegetables using a small and inexpensive Raman spectrometer are presented.

In Chapter 2, measurement and analysis methods for rapid analysis are examined. In this chapter, PMIR is proposed as a new spectral measurement and analysis method. PMIR is a method of measuring with a short exposure time so that objected peaks appear only in the spectra of a high-concentration sample and of determining from the peak-to-baseline ratio whether the concentration is high. PMIR cannot perform quantitative analysis, but it is specialized for the purpose of rapid concentration discriminant analysis using only a narrow wavenumber range. As a result of confirming the applicability of PMIR using vegetable juice samples with different carotenoid concentrations, a discrimination accuracy of 93% can be obtained in one-fifth of the measurement time of the conventional method.

In Chapter 3, the minimum required spectral performance is examined. In Raman spectrometers, the lower the wavenumber resolution and smaller the number of pixels in the detector, the smaller and cheaper the device can be developed. Therefore, a spectrum with reduced wavenumber resolution and number of pixels is computationally synthesized to simulate the accuracy of the quantitative and discriminant analyses. As a result, quantitative analysis in high signal-to-noise spectra yields the same accuracy, even when the wavenumber resolution and pixel count are reduced from the original

8-11 cm^{-1} and 512 to 64 cm^{-1} and 64. On the other hand, as a result of the discriminant analysis of rapidly measured spectra, the accuracy was reduced when the wavenumber resolution and the number of pixels were lowered; however, 90% of the discriminant accuracy was obtained even when they were lowered to 64 cm^{-1} and 64.

In Chapter 4, the selection of the optimum excitation wavelength is attempted. In Raman spectroscopy, the excitation wavelength greatly affects the measurement accuracy because it is related to the resonance Raman phenomenon, fluorescence intensity, and reabsorption of Raman scattered light. In this chapter, tomatoes were measured using excitation wavelengths of 532, 785, and 1064 nm, which are often used in Raman spectrometers, and the accuracy of quantitative analysis was compared to identify the optimum excitation wavelength. As a result, the strongest resonance Raman effect was obtained at 532 nm, but the Raman peak and carotenoid concentration were not proportional because Raman scattered light was reabsorbed by chlorophyll. Therefore, the excitation wavelengths in the near-infrared region (785 and 1064 nm) should be used.

In Chapters 5 and 6, non-destructive analysis of carotenoids are examined for intact tomato and spinach, respectively, based on the findings obtained in Chapters 2–4. As a result, highly accurate quantitative and discriminant analysis was possible with an excitation wavelength of 785 nm for tomato and 1064 nm for spinach. Using PMIR, individuals with high carotenoid concentrations could be identified with a hit rate of 94% or more with an exposure time of less than one-fifth of the conventional method. Furthermore, the simulation revealed that sufficient analysis accuracy can be obtained even if the wavenumber resolution and the number of pixels are reduced.

In conclusion, measurement and analysis methods for rapid analysis, spectroscopic performance for miniaturization and cost reduction, and optimum excitation wavelengths for non-destructive analysis of carotenoids in fruits and vegetables are investigated. From the results of this study, the development of carotenoid sensors for field analysis is expected in the future.

Contents

Abstract

Contents

Figure and table captions

Chapter 1: Introduction.....	1
1.1. Increasing demand on analysis of functional ingredients	1
1.2. The role of sensors in agriculture	2
1.3. Raman spectroscopy.....	4
1.4. Objective and outline of the research	10
Chapter 2: Development of PMIR	13
2.1. Introduction.....	13
2.2. Materials and methods.....	14
2.2.1. Raman spectrometer	
2.2.2. Raman spectral measurements	
2.2.3. PMIR analysis method	
2.2.4. PLSR analysis method	
2.3. Results and discussion	18
2.3.1. Spectra obtained in the highest SNR condition	
2.3.2. Lycopene concentration regression model	
2.3.3. Spectra obtained using short exposure times	
2.3.4. PMIR analysis of vegetable juice	
2.4. Conclusions.....	24
Chapter 3: Analysis of the spectroscopic performance of NIR-excited Raman spectrometer	25
3.1. Introduction.....	25
3.2. Materials and methods.....	28
3.2.1. Raman spectrometer	
3.2.2. Raman spectral measurements	
3.2.3. Composite spectra calculation	
3.2.4. PLSR analysis method	

3.2.5. PLS-DA analysis method	
3.3. Results and discussion	32
3.3.1. Composite spectra at an excitation time of 500 ms	
3.3.2. Composite spectra at an excitation time of 20 ms	
3.3.3. Result of PLSR	
3.3.4. Result of PLS-DA	
3.4. Conclusions.....	42
Chapter 4: Decision of excitation wavelength	43
4.1. Introduction.....	43
4.2. Materials and methods.....	44
4.2.1. Samples	
4.2.2. Raman spectral measurements	
4.2.3. Carotenoid concentration measurement	
4.2.4. PLSR analysis method	
4.3. Results.....	47
4.3.1. Total carotenoid content and lycopene content of tomatoes	
4.3.2. PLSR analysis results (532 nm-excited)	
4.3.3. PLSR analysis results (785 nm-excited)	
4.3.4. PLSR analysis results (1064 nm-excited)	
4.4. Discussion	56
4.4.1. Difference of Raman spectra	
4.4.2. Difference of the result of PLSR	
4.5. Conclusions.....	60
Chapter 5: Discriminant analysis of carotenoid-rich tomato.....	61
5.1. Introduction.....	61
5.2. Materials and methods.....	63
5.2.1. Samples	
5.2.2. Raman spectral measurements	
5.2.3. Carotenoid concentration measurement	
5.2.4. PLSR analysis method	
5.2.5. PLS-DA analysis method	
5.2.6. PMIR analysis method	
5.2.7. Composite spectra calculation	
5.3. Results and discussion	67

5.3.1. Raman spectra	
5.3.2. Analytical result of 785 nm-excited Raman spectra	
5.3.2.1. PLSR analysis results	
5.3.2.2. PLS-DA analysis results	
5.3.2.3. PMIR analysis results	
5.3.2.4. Compositing spectra results	
5.3.3. Analytical result of 1064 nm-excited Raman spectra	
5.3.3.1. PLSR analysis results	
5.3.3.2. PLS-DA analysis results	
5.3.3.3. PMIR analysis results	
5.3.3.4. Compositing spectra results	
5.4. Conclusions.....	85
Chapter 6: Discriminant analysis of carotenoid-rich spinach.....	86
6.1. Introduction.....	86
6.2. Materials and methods.....	88
6.2.1. Samples	
6.2.2. Raman spectral measurements	
6.2.3. Carotenoid concentration measurement	
6.2.4. PLSR analysis method	
6.2.5. PLS-DA analysis method	
6.2.6. PMIR analysis method	
6.2.7. Composite spectra calculation	
6.3. Results and discussion.....	91
6.3.1. Result of total carotenoid concentration and chlorophyll concentration	
6.3.2. Raman spectra of spinach	
6.3.2.1. Comparison of 532 nm, 785 nm, 1064 nm-excited Raman spectra	
6.3.2.2. 785 nm-excited Raman spectra	
6.3.2.3. 1064 nm-excited Raman spectra	
6.3.3. Analytical result of 785 nm-excited Raman spectra	
6.3.3.1. PLSR analysis results	
6.3.3.2. PLS-DA analysis results	
6.3.3.3. PMIR analysis results	
6.3.3.4. Compositing spectra results	
6.3.4. Analytical result of 1064 nm-excited Raman spectra	
6.3.4.1. PLSR analysis results	

6.3.4.2. PLS-DA analysis results	
6.3.4.3. PMIR analysis results	
6.3.4.4. Composited spectra results	
6.4. Conclusions.....	115
Chapter 7: Summary and conclusions	116
Acknowledgements	118
References	120