論 文 内 容 の 要 旨

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論文題目
NMR-based analysis and characterization of minor components in
Japanese persimmon (*Diospyros kaki*) cultivars
（NMR 法による日本柿の微量成分分析の検討及び微量成分による
品種特徴づけに関する研究）

Introduction

　Diospyros kaki, better known as the Japanese persimmon, is the most widely cultivated species of the *Diospyros* genus. Persimmon is one of the oldest food plants and its cultivation began in China more than 2000 years ago. Persimmons are recognized as an outstanding source of biologically active compounds related to many health benefits. However, there are only limited efforts devoted to investigate the metabolites in persimmon fruit compared to other fruits, such as apple, tomato, mango, etc. In addition, the investigations on persimmons were generally focused on certain selected metabolites such as nutritional antioxidants, carotenoid and polyphenols and the metabolic profiling are not applied to persimmon fruits for an overview of the chemical compounds.

　Metabolic profiling, also known as metabolomics or metabonomics, provides a detailed and comprehensive overview of food composition, which has been applied in food science for food component analysis, quality/authenticity assessment, consumption monitoring, and physiological monitoring in food intervention, and so on. There are several technologies for metabolic profiling, including mass spectrometry (MS), nuclear magnetic resonance (NMR) spectroscopy, capillary electrophoresis (CE) and high performance liquid chromatography (HPLC), etc. Among these techniques, NMR

spectroscopy represents a powerful technique for food analysis because of many advantages: (1) It provides a faithful overview of many components in a mixture solution; (2) It can be performed without any extraction, purification and chemical derivation of each component; (3) quantitative data can be obtained by integration relative to a standard component without calibration curves; (4) unknown or unexpected chemical components can be identified through appropriate two-dimensional (2D) NMR measurements. The present study characterized persimmon juice by NMR spectroscopy. Since the main components such as water and sugars in persimmon juice make the minor components undetectable or detected with very poor signal-to-noise (S/N) ratio due to the limitation of dynamic range, Broad Band WET method was developed to analyze persimmon juice as a new NMR method for detecting minor components in complex mixtures. Detailed signal assignments of persimmon juice (Taishu cultivar) were carried out using various 2D NMR spectra obtained with Broad Band WET or WET sequences and individual compounds in persimmon juice was quantitatively analyzed in the state of complex mixtures. Furthermore, the discrimination of different Japanese persimmon cultivars were performed by combining the signal patterns of minor components detected with Broad Band WET ^{1}H NMR spectroscopy with multivariate statistical analysis.

Broad Band WET — a new method to observe quantitatively minor components in foods

The pulse sequence of Broad Band WET method is a new sequence that incorporates WET sequence on the basis of NOESYPRESAT sequence. Broad Band WET spectrum was compared to the spectra collected with conventional saturation methods such as NOESYPRESAT, DPFGSE-WATERGATE and conventional WET. In the ^{1}H spectrum using NOESYPRESAT, the resonances from sugars were still strongly observed. As a result, signals in high-field region were detected with relatively poor S/N ratio of 517 for the signal of citric acid and few signals were observed in the low-field region. On the other hand, Broad Band WET sequence successfully suppressed not only the resonances of water but also those of sugars to very low levels. Minor components in the high-field region were observed with a good S/N ratio of 1998 and many peaks were observed in the low-field region. In addition, the saturation profile indicated a high

selectivity of saturation for the Broad Band WET sequence, which is very helpful in quantitative analysis because the concentrations of minor compounds can be estimated just by integrating the peak area relative to a standard reference at a known concentration. Furthermore, Broad Band WET method resolved the signal distortion that is caused by the effects of *J*-modulation in DPFGSE-WATERGATE method.

Spectral analysis of Japanese persimmon juice (Taishu cultivar)

A mixture analysis by one-dimensional (1D) NMR and a combination of several 2D NMR spectroscopy (^{1}H–^{1}H DQF-COSY, ^{1}H–^{1}H TOCSY, ^{1}H–^{13}C HSQC, and ^{1}H–^{13}C CT-HMBC) incorporating Broad Band WET sequence or WET sequence was carried out for the identification and quantification of chemical compounds in persimmon juice (Taishu cultivar). Because of the low sensitivity of ^{13}C NMR spectrum, the signals from the minor components cannot nearly be detected in both high-field region and low-field region, which made the assignment of minor components very difficult. To resolve this problem, the 10 mm probe was used to acquire ^{13}C NMR spectra, combined with condensed persimmon juice. As a result, the signals from minor components were observed clearly, and 26 compounds were identified.

The signals in the high-field region from 0.5 ppm to 3.0 ppm were derived from protons of free amino acids (alanine, citrulline, GABA, glutamine, glutamate, etc.) and organic acids (malic acid, citric acid, etc.). The signals at the low-field region from 5.6 to 11 ppm were derived from the aromatic protons of amino acids, uridine, adenosine, trigonelline, acetaldehyde and some singlet signals were assumed to be derived from polyphenols. In this region, the broad signals were assigned to the amino protons from glutamine and confirmed by spiking the standard compound. In addition, most components were quantified by the integration of signals using conventional ^{1}H NMR and Broad Band WET ^{1}H NMR spectra.