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学位論文名	Application of metabolomics techniques for the quality assessment of an oriental folk medicine, Angelica acutiloba roots （生薬トウキ（Angelica acutiloba）の品質評価におけるメタボロミクス技術の適用）
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論文内容の要旨

Treatment by traditional herbal medicine has been widely accepted for its effective therapeutic properties in remedying diverse diseases. One of the important traditional herbal materials is the dried roots of *Angelica acutiloba* (toki in Japanese) known for treating women's gynecological disorders, and for its potent immunomodulatory activities utilized in combination with conventional cancer therapy. Currently, the existing quality assessment method for its commercial product has become insufficient because a high-throughput protocol that adapts a holistic analysis of the entire complex chemical composition of the herbal material is ignored. Metabolomics is an interdisciplinary tool that includes a quantitative exhaustive profiling of all metabolites contained in a target organism through the use of high-throughput machines. Hence a non-targeted global analysis of the chromatographic patterns of various metabolites could be used to discriminate between samples of different status or origin via computational multivariate analysis (chemometrics), and interpret the trends that have a dominant affect in dissociating diverse samples. The application of metabolomics to the quality assessment of toki should prove to be a simple, fast and sufficient method.

In chapter 2, gas chromatography mass spectrometer (GC-MS) was utilized to elucidate the profiling of hydrophilic, low molecular weight compounds which includes primary metabolites. GC-MS is considered the most mature technology for analytical chromatography possessing high-throughput capability, sensitivity, and reproducibility. The differences between Yamato-toki with respect to quality determined by traditional method were rapidly processed and evaluated through principal component analysis (PCA). Distinct discrimination between good, moderate and bad qualities were shown where separation was influenced by the differences in cultivars and cultivation areas; the latter being the primary component for sample separation. This implicates the importance of cultivation area in affecting quality discrimination, and the degree of influences it possesses in dictating the global metabolite variations of Yamato-toki. Furthermore, PCA results signified two reducing

sugars as being the most accumulated in the bad quality, whereas significant quantities of specific organic and amino acids were found in high and moderate qualities.

In chapter 3, non-targeted metabolite fingerprinting of hydrophobic low molecular weight compounds in toki samples were analyzed by ultra performance liquid chromatography mass spectrometer (UPLC-MS). This analytical system has higher throughput, better separation efficiency and higher resolution in comparison with conventional HPLC. Accurate mass chromatographic fingerprints of positive and negative ESI modes were collected simultaneously, and compared through PCA analyses. Distinct partitioning of root samples was observed, in which cultivation area was also the major influential factor for sample disassociation. The affect of variety differences was diminutive to quality discrimination, and differences in rank could only be achieved when variability in cultivation area was eliminated. Henceforth, classification models by partial least square discriminant analysis (PLS-DA) were constructed in predicting quality based on cultivation area in which the results were reliable and accurate in categorizing test set samples. Further understandings to the chemical constituents in relations to quality discrimination were attained where some ion markers significantly linked to dissociation were tentatively identified as some subclasses of flavonoids and other secondary metabolites.

In chapter 4, the implementation of pyrolyser (PY) coupled to GC-MS was made in order to procure faster analysis of a broader spectrum of compounds, such as volatiles, low and high molecular weight compounds. Metabolite fingerprinting was employed making data analysis less tedious, less complex and less time-consuming. Furthermore, the combination of PY to the GC-MS analytical system eliminated sample preparation procedures, as raw samples of toki could be directly injected to the PY for thermal cracking or hydrolytic cleavage, and consecutively be analyzed by GC-MS. Hence, the chromatographic pattern attained from PY-GC-MS represents a more universal view of the global metabolite profile. With the introduction of metabolite fingerprinting to PY-GC-MS analysis, optimization to its functionality (analytical, practical and systematical aspects) was obtained, establishing its application as one of the useful QC methods.

As a general conclusion, the establishment of reliable, efficient and standardized quality assessment method for toki was successfully developed. Through the application of metabolomics technique, the influence of cultivation area to quality assessment was conceptualized and applied to the development of a legitimate QC method. The implementations of the three analytical platforms investigated have shown to have high robustness and reliability. Overall, PY-GC-MS was the ideal method that was developed for the quality assessment of commercialized toki products that can be applied to industrial fields. This is because PY-GC-MS maintains the GC-MS high-throughput capability, sensitivity and reproducibility while omitting sample preparation procedures and maximizing chemical universality. Hence, a more complete overview of the global metabolite fingerprints could be acquired in one single step where trained technician is not mandatory. The development of GC-MS-based metabolite profiling is well-suited for exploring the biological pathways of the herbal plant in the attempt to discover the optimal cultivation condition for yielding the best quality of toki raw material in agriculture. UPLC-based metabolomics would be most appropriate for pharmaceutical investigations where further elucidation to the therapeutic efficacy of toki roots and its correlation to quality assessment could be attained based on the fingerprints of secondary metabolites.

論文審査の結果の要旨

本論文は種々の質量分析法による生薬原料評価を目的とし、トウキにおける測定方法の検討を行い、新規のデータ解析方法の開発とその応用についてまとめたものである。

主な成果は以下の通りである。

（１）生薬ディーラーにより鑑定された製品トウキの品質を解析するために，ガスクロマトグラフィー質量分析法（GC/MS）の検討を行った．トウキサンプルの親水性代謝物を抽出し，シリル化等の誘導体化を行った後，GC/MS 分析を行った．得られた分析結果を保持時間インデックスを独立変数，質量分析強度を従属変数として，ベクトルに変換し，結果を行列データにまとめ主成分分析を行ったところ，トウキの起源，栽培加工地域による差異を表現することに成功した．

（２）上記に GC/MS による品質解析システムを開発したが，さらに，一般的な分析方法である HPLC の分析結果による品質解析システムの構築を検討した．トウキサンプルの有機溶媒抽出サンプルを用いて高速液体クロマトグラフィー飛行時間型質量分析を実施，保持時間インデックスを独立変数．質量分析強度を従属変数としてベクトルを作成し，結果を行列データにまとめ多変量解析を行った．LC/MS 分析結果を説明変数，栽培加工場所を応答変数として，PLS-DA 法により判別モデルの構築を試み，十分な予測力を有するシステムの開発に成功した

（３）上記で開発した方法は，有用ではあるが，煩雑な誘導体化処理あるいは，抽出処理を行う必要があった．そこで，より簡便な方法として，製品トウキを熱分解抽出し，発生した熱分解ガスを GC/MS 分析にかけ，得られたパイログラムによる品質予測システムの構築の検討を行った．保持時間インデックスを独立変数．パイログラムの質量分析強度を従属変数としてベクトルを作成し，結果を行列データにまとめ多変量解析を行ったところ，誘導体化を伴う GC/MS 分析結果と同等以上の品質でトウキの起源，栽培場所を判別することに成功した．本システムは，煩雑な誘導体化処理等の前処理が不要であり，極めて実用性の高い方法と考えられる．

以上のように，本論文は，複数の質量分析法を用いた生薬原料品質解析方法を提示し，それらの特徴を精査し，実用性を示している．よって，本論文は，博士論文として価値あるものと認める．