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学位論文名	Quality assessment of Japanese green tea, *Camellia sinensis*, by techniques in metabolomics （メタボロミクス技術を用いた緑茶（*Camellia sinensis*）の品質鑑定）
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論文内容の要旨

Novel techniques for Japanese green tea's quality assessment have been developed. By utilizing techniques in metabolomics along with chemometrics, chemical information could be extracted out from complex datasets.

The basic concept of this new approach is fast analysis by using chemical fingerprints instead of the characterization on a limited number of individual compounds. A number of analytical techniques were performed to explore metabolites containing in tea leaves. Hydrophilic-primary metabolites were analyzed by a coupled between gas chromatography and mass spectrometry (GC/MS). Subsequently, a system of ultra-performance liquid chromatography with mass spectrometry (UPLC/MS) was introduced to provide information of green tea's secondary metabolites. Finally, a system of hyphenated pyrolyzer-GC/MS was developed in order to omit sample's preparation step and explore entire metabolites comprehensively. Projection to latent structure by means of partial least-square (PLS) was performed to verify the correlation between green tea's metabolite profiling (X matrix) and its quality (Y matrix). A quality of model was validated by testing and comparing the predictive ability to the respective model.

Chapter 1: General introduction

Quality of tea is different in kind of tea tree, plucking time, cultivation method and post harvest treatment. These variations lead to differences in metabolites content in tea leaves, thus a variation in tea's quality. Sensory evaluation of Japanese green tea's quality has traditionally been assessed by highly trained specialists who evaluate product quality on the basis of leave's appearance, aroma, color and taste of the brew. Besides personal proficiency, it takes several years of specialized training to become a professional tea taster. Recently, many instrumental measurements and analysis have been introduced to evaluate and determine the quality of product by focusing on specific group compounds. However, the author believes that characteristics of product result from combination of overall metabolites rather than a single compound. Therefore, techniques in metabolomics were in the consideration.

Chapter 2: Quality-predictive model by GC/MS based primary metabolite fingerprinting

Primary metabolites of green tea, which are believed to be key factors to tea's taste, were investigated. Green tea flavor has four characteristic taste elements: bitterness, astringency, sweetness and umami. The brothy, sweet umami taste is mainly due to amino acids. A relationship between green tea's metabolite profile and its quality was explored. A combination of gas chromatography and mass spectrometry (GC/MS) allows the identification and quantification of numerous metabolites within single extract. A quality-predictive model based on green tea's primary metabolite profile was constructed through PLS regression describing 82.2% of variations in Y and predicting 50.0% of variations in Y. Quinic acid, group of amino acids and sugars were found to be significant in creating the prediction model.

Chapter 3: Quality-predictive model by UPLC/MS based secondary metabolite fingerprinting

Subsequently, ultra-performance liquid chromatography coupled with mass spectrometry (UPLC/MS) was selected as an analytical platform providing information of green tea's secondary metabolites. Several researches show that tea contains a large number of plant secondary metabolites such as catechins, purine alkaloids, flavonoids, etc. Several of them are demonstrated to be an important quality parameter determining price of product. Green tea with different quality was discriminated. Consequently, projection to latent structure by means of PLS was performed. The green tea's quality-predictive model described 83.8% of variations in Y and predicted 71.4% of variations in Y. Compounds found to have high relevance to variation in tea's quality were group of polyphenols.

Chapter 4: Quality-predictive model based overall metabolites by PY-GC/MS

Finally, a fast, simple and low-cost approach to evaluate quality of green tea was achieved by a system of hyphenated pyrolyzer-GC/MS. The error from sample preparation could be avoided since raw samples were extracted through pyrolyzer. In addition, undesired reactions from costly derivatizing agents, which are commonly needed to treat samples before GC/MS analyses could be ignored. A quality-predictive model by means of PLS regression was created and yielded a good model with high value of goodness and fitness of the model. The model could describe 87.6% of variations in Y, while predict 77.8% of variations in Y. Differences in product's quality were resulted from content of phenolic-derived compounds and also group of long-chain hydrocarbons.

Chapter 5: Conclusion

Techniques for quality evaluation of Japanese green tea were successfully developed by means of metabolic fingerprinting utilizing spectroscopic data along with multivariate analysis. By observation of all metabolites containing in tea leaves, the complete information of the system was acquired.

Synopsis of Evaluation Results

The thesis comprises three academic publications; two publications have been published and the other one has been submitted. The oral examination was held on 30th June 2008 and the professor committee has agreed that the examination and the thesis have met the requirements for the applicant to receive the degree of Doctor of Philosophy in Engineering.

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食品の二次機能の評価を目的とし、緑茶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）奈良県煎茶品評会で鑑定された製品緑茶の品質を鑑定するために，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．鑑定順位付き緑茶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多変量解析を行った．GC/MS 分析結果を説明変数，品評会鑑定順位を応答変数として，PLS 法により順位予測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．本システムは，熟練した官能試験しに依存していた官能試験に代わるシステムとして極めて有用である．
（2）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を行う必要があった．そこで，より簡便な方法として，製品緑茶を熱分解抽出し，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．GC/MS 分析結果を説明変数，品評会鑑定順位を応答変数として，PLS 法により順位予測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．本システムは，煩雑な誘導体化処理が不要であり，極めて実用性の高い方法と考えられる．

（３）上記に GC/MS による予測システムの開発結果を示したが，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予測システムの構築を検討した．緑茶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，品評会鑑定順位を応答変数として，PLS 法により順位予測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

以上のように，本論文は，複数の質量分析法を用いた食品品質鑑定方法を提示し，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．