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Quality evaluation of *Angelica acutiloba* Kitagawa roots by ^{1}H NMR-based metabolic fingerprinting

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ABSTRACT

The prices of *Angelica acutiloba* Kitagawa (yamato-toki) and *A. acutiloba* Kitagawa var. *sukiyamae* Hikino (hokkai-toki) are now mainly determined according to the sensory quality determined by experts in addition to the physical properties. This method provides a low reliability result for differentiating and qualifying their qualities. In addition, the quality in terms of pharmacological efficiency is not taken into account for consideration in the ordinary sensory method. A combination of a ^{1}H NMR technique and a multivariate analysis was preliminarily applied for the quality evaluation of both toki roots with regard to their geographical and variety differences. A broad range of metabolites was detected by a single-run ^{1}H NMR spectrometry. Partial least-squares discrimination analysis (PLS-DA), a pattern recognition method, was applied to the ^{1}H NMR spectra of aqueous extracts of toki samples having different sensory qualities. The PLS-DA result showed a clear clustering corresponding to the cultivation area between toki samples cultivated in Hokkaido (Japan) and those cultivated in the southern part of China and the Nara prefecture (Japan), while there was no separation corresponding to the toki's variety and sensory qualities, indicating the inconsistency of the sensory evaluation result. The chemical metabolites contributing to the discrimination of toki samples in relation to pharmacological and sensory properties were reported for the first time. A reliable multivariate calibration model used to predict the sensory quality was successfully carried out by PLS regression.

1. Introduction

Traditional Chinese Medicine (TCM) has been widely used for several centuries in Asian countries including China, Korean, and Japan because of its therapeutic effects. The roots of *Angelica sinensis*, *Angelica gigas* Nakai, and *Angelica acutiloba* Kitagawa (yamato-toki in Japanese) as well as *A. acutiloba* Kitagawa var. *sukiyamae* Hikino or hokkai-toki, a different variety of yamato-toki, have traditionally been used in the treatment of gynecological diseases such as menoxenia, arthritis, and anemia because of their hematopoietic, analgesic, and sedative effects [1–3]. However, these *Angelica* roots exhibit variations in their chemical constituents and pharmacological effects according to the differences in species, geographical conditions, as well as processing methods [4,5]. A high-performance liquid chromatography study revealed that the chemical compounds in *A. sinensis* and *A. acutiloba* were rather different in that the latter contained a high amount of coniferyl ferulate [6] while the former contained ferulic acid and *Z*-ligustilides [4]. Recently, a gas chromatography/mass spectrometry pattern recognition method revealed that decursin and decursinol angelate contributed the most toward distinguishing *A. gigas* from *A. sinensis* and *A. acutiloba* [5].

Ferulic acid and ligustilide are the major chemical constituents that are directly related to the pharmacological activity of *Angelica* roots; therefore, they were generally used as chemical markers for the quality assessment of the *Angelica* roots, especially in the case of *A. sinensis* [4,7,8]. The former compound had an inhibitory effect on platelet aggregation and serotonin release, while the latter exhibited the anti-asthmatic and spasmolytic effects [9–11]. It has been reported recently that in addition to ferulic acid, *Z*-butylidenephthalide can also be used as a chemical marker to monitor the quality of *A. sinensis* [12].

As mentioned above, the quality of the *Angelica* roots depended on several factors including geographical origins. Many attempts have been made by means of chromatographic and spectroscopic techniques to study the effect of geographical conditions on the differences in pharmacological chemical constituents in *Angelica* roots [2,5,6,13–16]. However, the quality of *Angelica* roots in terms

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of pharmacological efficiency is only one of the factors that is considered in the commercial Japanese markets wherein many characteristics are considered to judge the quality of the roots.

The price of crude *A. acutiloba* roots (yamato- and hokkai-tokis) mainly depends on their sensory qualities; these are judged by experts based on the appearance, aroma, and taste of the roots. Here, it should be noted that only the few compounds of interest cannot be used for evaluating the sensory quality of the complex samples. Therefore, the use of only ferulic acid or ligustilide as a preliminary marker compound to describe the quality of the crude sample appears to be inadequate. In addition, this ordinary evaluation method is inconsistent in differentiating or grading the quality of crude samples in commercial markets.

Analytical techniques including mass spectrometry (MS), high-performance liquid chromatography (HPLC), and nuclear magnetic resonance (NMR) spectrometry detect a wide range of metabolites resulting in complex data sets. Among these techniques, NMR is considered to be the most suitable technique for the purposes of quality evaluation and differentiation due to its nondestructive nature and non-selectivity, along with the fact that it reveals a significant amount of structural information; further, the use of NMR simplifies the process of sample preparation and decreases the time required for analysis. This information, in combination with the chemometric information obtained by pattern recognition techniques such as partial least-square projections to latent structures-discrimination analysis was expected to provide reliable and conclusive information about the chemical compositions in crude samples in terms of their sensory qualities. In addition, PLS regression was suggested to offer a highly accurate predictive model of these roots with regard to their sensory qualities.

In this study, a combination of non-selective ^{1}H NMR-based metabolomics and a multivariate analysis by PLS-DA was performed to discriminate toki samples having different sensory qualities with regard to geographical and variety differences. PLS regression was later employed to build a quality predictive model to predict the sensory qualities of an unknown toki sample.

2. Materials and methods

2.1. Materials

Six graded samples of crude dried yamato-toki and one sample of dried hokkai-toki roots obtained from the Fukuda shoten Company, Japan, were used in this study. Their characteristics are listed in Table 1. The first, second, and last alphabet letters in a sample name represent the toki's variety, cultivation area, and sensory quality, respectively. The sensory qualities of these samples were judged by a professional taster from the Fukuda shoten Company who has vast expertise and experience in sensory evaluation.

2.2. Chemicals and reagents

All standard compounds used for ^{1}H NMR assignments were analytical grade with purity higher than 90%. Deuterium oxide (D_2O, D 99.9 atom%) purchased from Cambridge Isotope Laboratories, Inc., and 3-(trimethylsilyl)-1-propanesulfonic acid, sodium salt (DSS, 97%) obtained from Aldrich were used as the solvent and internal standard, respectively, for all ^{1}H NMR measurements. A phosphate buffer solution (1.0 M, pH 7.4) obtained from Sigma was also used in this experiment.

2.3. Sample preparation for ^{1}H NMR analysis

1.5 mL of D_2O was added to 150 mg of dried toki roots in a 2-mL Eppendorf tube. The mixture was continuously incubated and shaken in a Thermomixer comfort (Eppendorf) at 70 °C and 1400 rpm for 3 h, followed by centrifugation at 25 °C and 15,000 rpm for 30 min (TOMY MX-150). 300 μL of supernatant containing hydrophilic metabolites was then transferred and mixed with 300 μL of 0.2 M phosphate buffer solution containing 3 mM DSS to give a 600-μL solution for NMR measurement. All samples were prepared in 1 day and stored at 4 °C prior to the analysis.

2.4. NMR spectrometry

^{1}H NMR spectra were recorded at 25 °C using a 750-MHz Varian Inova 750 spectrometer using a 5-mm ^{1}H{^{13}C/^{15}N} triple resonance indirect detection probe. D_2O and DSS were used as the internal lock signal and internal standard at chemical shift (δ) 0.0 ppm, respectively. The ^{1}H NMR measurement was carried out with 64 transients and 128K complex data points. The acquisition time and recycle delay were 6.257 and 3.743 s per scan, respectively, using a 30° pulse angle. The water suppression enhanced through the T1 effects (WET) pulse sequence was applied to suppress a water signal. All spectra were Fourier transformed with 0.1-Hz line broadening prior to data reduction and preprocessing.

2.5. NMR data reduction and preprocessing

All NMR spectra were first phase adjusted and baseline corrected by Chenomx NMR Suite 4.6 software, professional edition (Chenomx Inc., Canada). Each NMR spectrum was bucketed by integrating the regions having an equal bin size of 0.01 ppm over a range of δ 0.7–8.5 ppm, while those of a water signal between δ 4.5 and 5.1 ppm were eliminated. All bins were normalized to the total peak area to provide the absolute contributions of particular resonances to the spectrum prior to the conversion of the data from the Chenomx software format to the Microsoft Excel format (*.xls). The Excel format was then imported into Simca-P software, Version 11 (Umetrics AB, Umeå, Sweden) for multivariate analysis.

2.6. ^{1}H NMR assignment and pattern recognition

The chemical shifts of the significant chemical constituents were assigned by comparing their resonances to the Chenomx NMR Suite 4.6, 800 MHz (pH 4–9) library database provided by Chenomx Inc., and the in-house library.

Partial least-squares discrimination analysis (PLS-DA), a supervised pattern recognition method, of the ^{1}H NMR spectra was performed using Simca-P software, Version 11. This approach is used in order to explain the separation among groups of observations. It determines the relationship between a set of latent variables corresponding to principal components in principal component analysis (PCA) and class matrices, and describes the variable information that significantly affects the class separation [17]. In this analysis, the mean center was used as a preprocessing method.

2.7. ^{1}H NMR linear regression model by PLS regression

PLS regression is a PLS approach that generalizes and combines features from PCA and multiple regression. This approach relates two data matrices, *X* and *Y*, by a linear multivariate model. This model attempts to find a multidimensional direction in the *X* space that explains the maximum multidimensional variance direction in the *Y* space. It is used to predict a dependent variables data set from a large set of independent variables and can be used to analyze data with noisy and incomplete variables in both matrices [18,19]. In this study, PLS regression was performed

Table 1
Identification and quality classification of toki roots obtained from Fukuda shoten, Japan

Samples	Species	Cultivation area	Quality ranking (sensory result)
YNA	*A. acutiloba* Kitagawa	Nara prefecture	A
YNB	*A. acutiloba* Kitagawa	Nara prefecture	B
YNC	*A. acutiloba* Kitagawa	Nara prefecture	C
YHC	*A. acutiloba* Kitagawa	Hokkaido	C
YCD	*A. acutiloba* Kitagawa	Southern part of China	D
YCE[a]	*A. acutiloba* Kitagawa	Southern part of China	E
HHE[b]	*A. acutiloba* Kitagawa var. *sugiyamae* Hikino	Hokkaido	E

The first letter "Y" and "H" denotes yamato-toki and hokkai-toki, respectively.
[a] YCE was judged to have the worst quality, but higher than HHE.
[b] HHE was judged to have the worst quality among toki samples.

using the Simca-P software, Version 11, using ^{1}H NMR data in a region between δ 0.78 and 4.35 ppm, and unit variance (UV) as a preprocessing method.

3. Results and discussion

3.1. Identification of chemical constituents in crude dried yamato-toki roots

All yamato- and hokkai-toki roots were extracted using D_2O at 70 °C for 3 h in order to gather all hydrophilic constituents used in the quality evaluation. The chemical metabolites were almost identical for all samples; however, variations were observed in the chemical contents depending on the quality. An aqueous extract of the best sensory quality yamato-toki (YNA) was characterized and shown in Fig. 1. The resonances of the metabolites were assigned by comparing them with the signals of the Chenomx NMR Suite 4.6, 800 MHz (pH 4–9) database and the in-house library measured under the same conditions as the toki samples. The corresponding resonances were in good agreement with the standard assignment. However, some resonances differed slightly from those assigned in the Chenomx NMR database due to the differences in the measurement condition. Approximately 20 compounds including sugar, organic compounds, amino acids, alkaloids, and coumarin were identified.

Sugar compounds were mostly observed in the region between δ 3.00 and 5.50 ppm. Sucrose was the major disaccharide (non-reducing sugar) in toki roots having resonances at δ 3.46, 3.55, 3.67, 3.75, 3.80, 3.84, 3.88, 4.04, 4.20, and 5.40 ppm. Lactose, another disaccharide constituent, was detected at δ 3.27 and 3.65 ppm. Signals due to monosaccharides (reducing sugar) including fructose and glucose clearly resonated at δ 3.69, 3.95, and 4.0 and 3.23, 3.46, 3.55, and 5.22 ppm, respectively. Several organic compounds including malate (δ 2.37, 2.64, 2.68, and 4.30 ppm), citrate (δ 2.47 and 2.56 ppm), 4-aminobutyrate (δ 2.28 and 3.00 ppm), fumarate (δ 6.50 ppm) and maleate (δ 5.98 ppm) were also detected. Some small signals expected due to ferulic acid (δ 6.89, 7.68, and 7.17 ppm) and its derivative ferulate (δ 6.89, 7.18, 7.23, and 7.32 ppm), which are normally used as indicators of the pharmaceutical quality of *Angelica* roots [7], resonated in the high-frequency region between δ 6.50 and 8.00 ppm.

Seven types of amino acids including arginine (δ 1.64, 1.71, 1.90, 3.23, and 3.75 ppm), proline (δ 1.99, 2.05, 2,34, 3.33, 3.40, and 4.12 ppm), alanine (δ 1.46 ppm), valine (δ 0.97 and 1.03 ppm), threonine (δ 1.31 ppm), leucine (δ 0.93 ppm), glutamine (δ 2.13 and 2.44 ppm), asparagine (δ 2.84 and 2.95 ppm), and phenylalanine (δ 7.32, 7.36, and 7.42 ppm) were detected. In addition, caffeine (δ 3.35 ppm), an alkaloid compound, as well as umbelliferone (δ 6.88, 7.54, and 7.98 ppm), a type of coumarin derivative, were also detected in the toki roots.

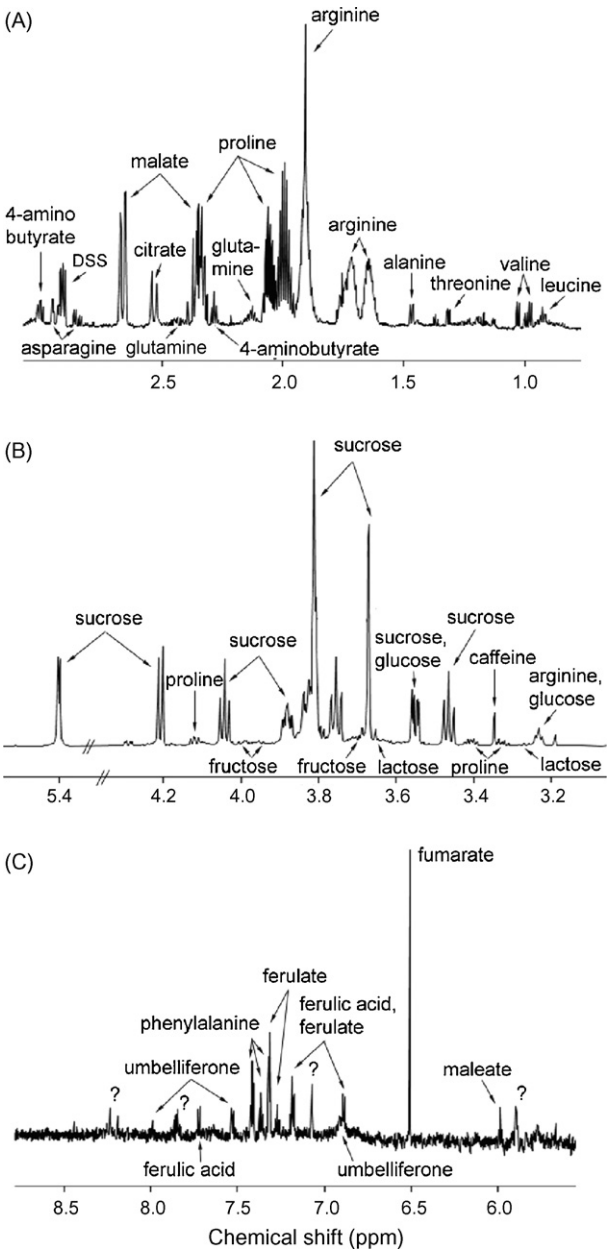

Fig. 1. ^{1}H NMR spectra (750 MHz, D_2O) of toki extract from the highest quality sample (YNA) in (A) high- (B) middle-, and (C) low-frequency regions, measured at 25 °C.

3.2. Metabolite profiling and fingerprinting of toki aqueous extracts: relationship of cultivation origin, toki's variety and chemical profile

Fingerprinting avoids the problems inherent in complicated signal assignments. Instead, multivariate analysis is used to compare sets of spectra by sorting data sets into categories without overall metabolites assignments. The outcome can be inferred to the discrimination among groups of related samples and the profiling can also be used to compare and identify the significant differences in the concentrations of metabolites [20]. In this study, PLS-DA, a supervised pattern recognition method was used to elucidate the discrimination among toki samples cultivated in different areas. It was applied to six yamato-toki (YNA, YNB, YNC, YHC, YCD, and YCE) and one hokkai-toki (HHE) samples. YNA, YNB, and YNC were cultivated in the Nara prefecture in Central Japan, while YCD and YCE were grown in Southern China. HHE was grown in Hokkaido, Northern Japan. The sensory quality of the toki (yamato-toki and hokkai-toki) judged by the experts based on a sensory test was ranked from A to E in which the best quality was assigned as A and the worst as E grade, denoted by the last letter of each sample. The results of the PLS-DA score and loading plots of their hydrophilic extracts measured in a ^{1}H NMR region between δ 0.7 and 8.5 ppm, after subtraction of the residual water signal, are shown in Fig. 2A and B, respectively.

The PLS-DA score plot showed the obvious clustering of toki samples in terms of their cultivation region in the first and second PCs. The toki samples grown in Hokkaido (HHE and YHC) were clearly separated from those cultivated in Nara (YNA, YNB, and YNC) and China (YCD and YCE) along the PC1 axis, whereas no significant separation was observed between those cultivated in Nara and China. The corresponding loading plot of PC1 in Fig. 2B showed that toki cultivated in Hokkaido contained a high amount of caffeine, lactose, and reducing sugar (glucose and fructose), as well as a small amount of proline and malate; those grown in Nara and China mainly comprised arginine and sucrose. The difference in the chemical constituents between toki grown in Hokkaido and those in Nara and China was expected due to the difference in the sample preparation procedure, wherein all yamato-toki samples were washed with hot water prior to the drying process, whereas hokkai-toki was dried without hot-water extraction. Another possibility was the difference in the cultivation area as well as the climatic condition. However, the former possibility was neglected considering the fact that no clear separation was observed between hot-water-extracted yamato-toki grown in Hokkaido (YHC) and unwashed hokkai-toki (HHE). By considering the yamato-toki samples, it is remarkable that the yamato-toki grown in Hokkaido, YHC, exhibited a different metabolite profiling as compared to those grown in Nara and China. In contrast, no significant difference was observed in the metabolites between YHC and HHE. These results demonstrated that the cultivation region played the most important role in terms of differences in metabolite constituents as compared to the preparation procedure and toki variety. The high proportions of reducing sugars and organic compounds in toki samples cultivated in Hokkaido, which has a cold climate, were in good agreement with a previous study on medicinal plants [21]. The effect of cultivation areas on the difference in the metabolite constituents could be described by the difference in the root respiration rates with regard to the change in climatic temperature. The root respiration rate was lower in plants cultivated in cold weather as compared to that in plants grown in a hot area [21]. Additionally, the contents of reducing sugars, organic and inorganic compounds were negatively correlated to the root respiration rate. Therefore, at lower temperatures, the roots respiration rate decreased and hence there was an increase in the proportions of reducing sugar and organic and inorganic constituents [21,22].

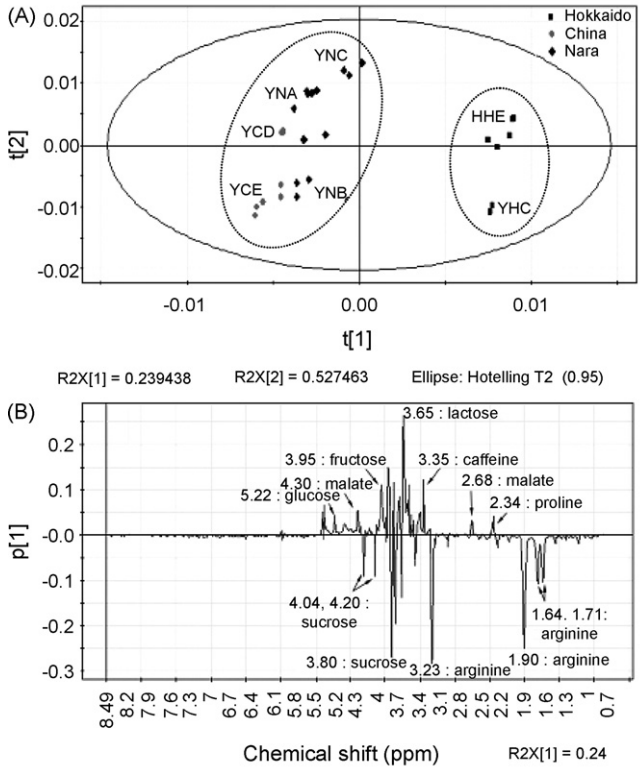

Fig. 2. PLS-DA of yamato- and hokkai-toki NMR profiles in the frequency region between δ 0.7 and 8.5 ppm, excluding the water region between δ 4.5 and 5.1 ppm. PLS-DA exhibits a clustering among toki samples cultivated in the Nara (♦), China (●), and Hokkaido (■) areas. (A) PLS-DA score plot of the first and second PCs and (B) PLS-DA loading plot of the first component responsible for PLS-DA classification.

The effect of the sensory quality on the discrimination of different toki samples cultivated in different climatic areas was investigated, and it is shown in Figs. 2 and 3. Toki samples cultivated in Hokkaido were significantly differentiated from those grown in Nara and China. In contrast, no clustering was observed in terms of the sensory quality ranking, as shown in Fig. 2A, which showed an adulterated result among the different sensory quality toki samples. However, the toki samples cultivated in Nara could be clearly distinguished in terms of the sensory quality from those harvested in China in the case of those grown in a cold climatic area; Hokkaido was eliminated. The corresponding PLS-DA score and loading plots are shown in Fig. 3A and B, respectively. Fig. 3A showed a clear classification between the best- to middle-quality toki samples (A–C grades) and the middle- to worst-quality ones (D and E grades) along the PC2 axis. These results demonstrated that the climatic condition had a strong influence on the sensory qualification result; it is suggested that these conditions affected the chemical constituents used by the professional taster as the key compounds in justifying the sensory quality.

By considering the corresponding loading plot shown in Fig. 3B, toki samples cultivated in Nara having a high sensory quality exhibited a positive correlation on PC2, whereas those grown in China having a lower quality had a negative score. The higher-quality toki samples mainly comprised sucrose and caffeine, while the lower-quality ones were rich in arginine indicating that sucrose and caffeine were the contributive metabolites in distinguishing the high sensory quality toki samples from the lower-quality ones. The positive relationship between these two contributive metabolites and the sensory quality appeared to confirm that these two

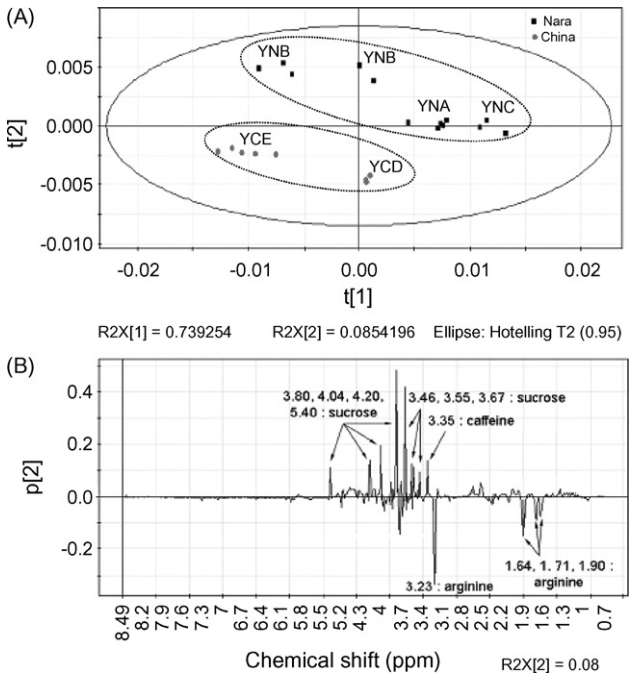

Fig. 3. PLS-DA of yamato-toki NMR profiles in the frequency region between δ 0.7 and 8.5 ppm, excluding the water region between δ 4.5 and 5.1 ppm. PLS-DA exhibits a clustering between yamato-toki cultivated in the Nara (■) and China (●) areas. (A) PLS-DA score plot of the first and second PCs and (B) PLS-DA loading plot of the second component responsible for PLS-DA discrimination.

compounds were the key constituents used by professional tasters in judging the quality of toki samples in the sensory test. As mentioned previously, many characteristics are considered to judge the sensory quality and thus the different origins of toki roots are expected to lead to different distinctive characteristics. Based on the above findings, the sweetness of high-quality toki was suggested to be caused by sucrose. In contrast, caffeine was expected to be the key constituent contributing toward bitterness, as it was reported in the quality evaluation of green tea [23]. However, it should be noted that excess caffeine content may probably reflect inversely on the quality, as in the case of HHE that contained a high amount of caffeine but was determined to have the worst sensory quality.

3.3. *Metabolite profiling and fingerprinting of toki aqueous extracts: relationship of sensory evaluation and pharmaceutical qualification*

The relationship between the sensory qualification and pharmacological property in terms of the cultivation area was further determined by the PLS-DA analysis of the ^{1}H NMR profiles after excluding the predominant sugar signals in the range from δ 2.8 to 5.5 ppm. Bioactive metabolites such as ferulate and ferulic acid resonated at the high-frequency region between δ 5.5 and 8.5 ppm. However, the sugar signals were several orders of magnitude larger than the signals from these metabolites. Therefore, the removal of sugar signals was necessary in the NMR spectra to overcome the dynamic range problem and enhance the intensities of the metabolites of interest. In the PC1/PC2 score plot shown in Fig. 4A, a clear separation between the toki cultivated in Hokkaido and those grown in Nara and China was observed along the PC1 axis. The corresponding loading plot of PC1 shown in Fig. 4B exhibits a positive correlation to arginine, 4-aminobutyrate, citrate, maleate, and fumarate, and a negative correlation to proline. The metabolites corresponding to the positive loading value were predominant in the toki samples cultivated in Nara and China, while those corresponding to the negative value were rich in the toki cultivated in Hokkaido. However, the signals due to ferulate and ferulic acid, which are usually used as the biomarkers for the evaluation of pharmacological quality [7], were not present in the discrimination result. This was expected to be due to the irrelevant concentration differences among toki samples, even in the case of those grown in different cultivation areas; hence, it is difficult to verify the correlation between the sensory quality and pharmacological bioactive constituents.

Therefore, as a preliminarily study, only the best to middle sensory quality toki samples cultivated in Nara were investigated and subsequently analyzed using PLS-DA. The resulting PLS-DA score plot is shown in Fig. 5A, showing a distinctive clustering between the best sensory quality toki (YNA) and those of the lower-quality ones (YNB and YNC) with respect to PC1, accounting for 63% of the total variables. YNA was characterized by a positive loading value comprising amino acids, organic compounds, and a small amount of ferulate, while YNB and YNC were defined by a negative value of the loading plot, as shown in Fig. 5B. As mentioned previously, ferulate is a principle biomarker that is used to evaluate the pharmacological property of toki samples wherein a high ferulate content corresponds to better pharmacological properties. The positive correlation between the presence of ferulate and the sensory quality implied that the quality in terms of pharmaceutical activity could be described in accordance with the sensory grading. With regard to this result, it was suggested that the quality of toki judged by a professional taster could be now described either for sensory or oblique pharmacological properties, although the latter property was not a predominant characteristic that professional tasters mainly consider when determining the price in the commercial market.

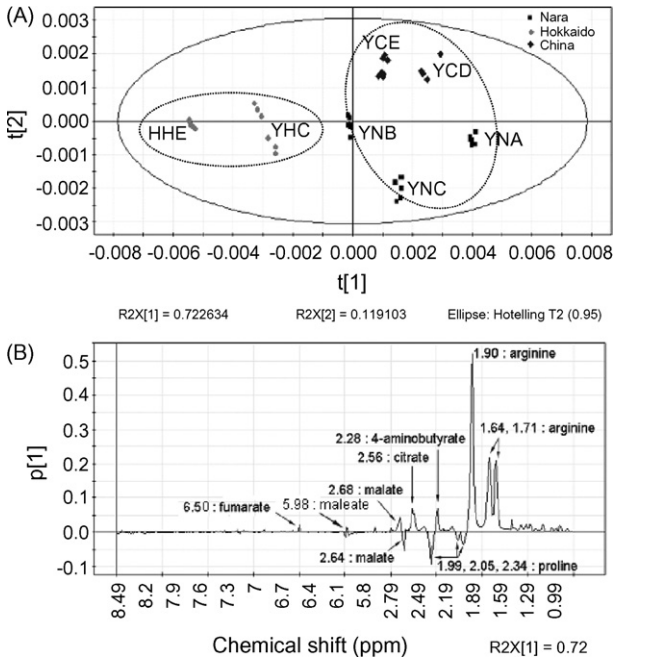

Fig. 4. PLS-DA of yamato- and hokkai-toki NMR profiles in the frequency region between δ 0.7 and 8.5 ppm by excluding the water and sugar region between δ 2.8 and 5.5 ppm. PLS-DA exhibits a clustering among toki samples cultivated in the Nara (■), Hokkaido (●) and China (♦) areas. (A) PLS-DA score plot of the first and second PCs and (B) PLS-DA loading plot of the first component responsible for PLS-DA discrimination.

3.4. Quality-predictive model by PLS regression

As mentioned previously, the sensory evaluation method is characterized by low reliability and inconsistency, and it is time consuming. Thus, a combination between non-targeted ^{1}H NMR data and chemometric information obtained by the PLS regression approach was proposed to provide a highly reliable and accurate prediction model for evaluating the sensory quality.

The regression model can potentially be used for predicting the dependent (response) variables Y from the independent (predictor) variables X [18,19,24]. The mathematical model is first proposed based on the system behavior, followed by a calibration step in which the optimal values for the model parameters corresponding to the training samples are determined. The values of unknown independent variables are then predicted in the prediction step by using the resulting training model [24].

In this study, the PLS regression was used to relate the variations in the NMR spectra in the region of δ 0.78–4.35 ppm to the sensory quality variation of the toki samples, and was applied to the sensory quality prediction of two different toki varieties. The sensory quality ranking was selected as a variable index (response variable). The entire data sets were divided into two parts: training and validation sets that were used to create the prediction model and verify the model's predictability, respectively. A correlation coefficient, R^2, and a cross-validated correlation coefficient, Q^2, as well as the validation error given as the root mean square error of the prediction, RMSEP, are usually used to verify the quality of the regression model. Generally, R^2, describing how well the data of the training set is mathematically reproduced, varies between 0 and 1, where 1 implies a perfect fit. Whether or not a prediction model is good can be judged in terms of the Q^2 value, and a model is considered to be good if $Q^2 > 0.5$; if $Q^2 > 0.9$, a model is considered to have an excellent predictive ability [25].

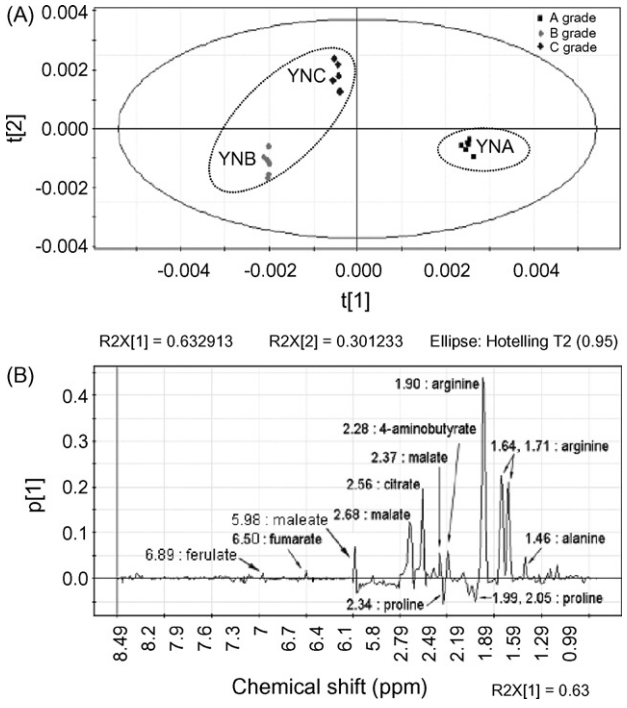

Fig. 5. PLS-DA of yamato-toki cultivated in Nara prefecture. NMR profiles in the frequency region between δ 0.7 and 8.5 ppm by excluding the water and sugar region between δ 2.8 and 5.5 ppm. PLS-DA exhibits a clustering of yamato-toki with different quality: A grade (■), B grade (●), and C grade (♦). (A) PLS-DA score plot of the first and second PCs and (B) PLS-DA loading plot of the first components responsible for PLS-DA discrimination.

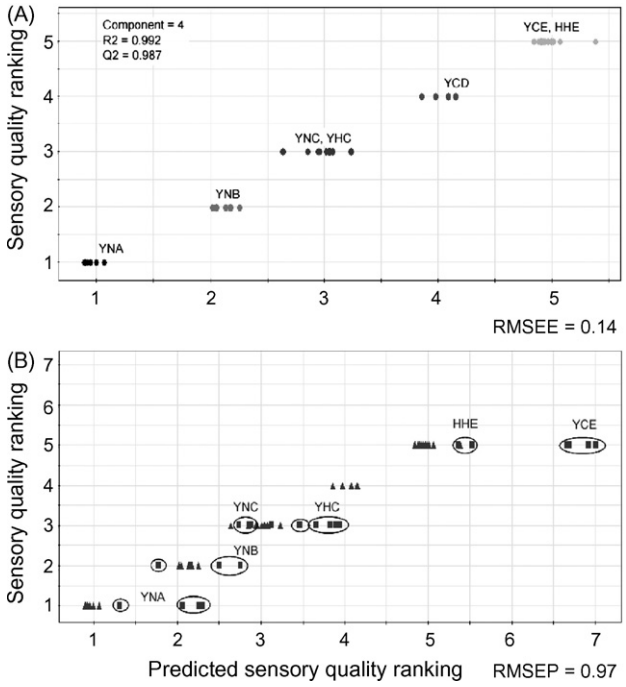

Fig. 6. Relationship between observed and predicted toki quality ranking for the PLS model calculated from ^{1}H NMR data in a region of δ 0.78–4.35 ppm: (A) regression model without validation set and (B) regression model with validation samples; training (▲) and validation (■) samples.

The PLS relationship between the measured and predicted sensory quality values of toki samples is shown in Fig. 6A. Four to six replicated ^{1}H NMR spectra of all toki samples were used to build the regression model. The data was preprocessed by a UV algorithm without the application of any scaling and transformations. The PLS regression analysis yielded the correlation coefficient describing the accuracy of prediction, $R^2 = 0.992$, and a cross-validated correlation coefficients indicating the model predictability, $Q^2 = 0.984$, as well as the root mean square error of the estimation, RMSEE = 0.14. The very high R^2 and Q^2 values clearly indicated the excellence of regression fitting and the predictability of the PLS model, respectively.

The capability of the model to predict the sensory quality of toki samples was further evaluated by subsequently applying the resulting PLS regression to the validation sets, which were triplicate measured, as shown in Fig. 6B. With the validation set, the prediction result was well plotted with respect to the diagonal regression having a validation error, RMSEP, of approximately 0.97. The smaller RMSEP value as compared to RMSEE, along with the very high R^2 and Q^2 values signified that this multivariate calibration could potentially be used for predicting the sensory quality of toki samples with very good accuracy and excellent predictability.

4. Conclusion

^{1}H NMR-based metabolomics provided useful details for the quality assessment of toki roots within one single-measurement wherein specific key metabolites were focused on for specific purposes; this method differed from that used in previous studies. The pattern recognition analysis by PLS-DA was successfully applied to investigate the impact of cultivation area, variety, sample preparation procedure, and sensory quality on the significant differences in metabolite contents. ^{1}H NMR fingerprinting and profiling revealed that the cultivation region was more significant than other factors in terms of the effect on metabolite differences. It was also found

that the climatic condition had a strong influence on the sensory qualification result since it affected some metabolites that contributed to the sensory properties. In addition, the PLS-DA result indicated a positive correlation between the sensory and pharmacological qualities wherein the best sensory quality toki contained the highest content of ferulate, a pharmacological bioactive constituent. The predictive model was also successfully constructed by using the PLS regression approach. It offered a very high reliability with excellent predictability for the sensory quality prediction of toki samples. With regard to these findings, a combination of metabolomics and multivariate analysis was then expected to be one of the best methodologies for the qualitative and quantitative evaluation of toki quality.

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