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Quality Evaluation and Prediction of *Citrullus lanatus* by ^{1}H NMR-Based Metabolomics and Multivariate Analysis

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^{1}H NMR spectrometry in combination with multivariate analysis was considered to provide greater information on quality assessment over an ordinary sensory testing method due to its high reliability and high accuracy. The sensory quality evaluation of watermelon (*Citrullus lanatus* (Thunb.) Matsum. & Nakai) was carried out by means of ^{1}H NMR-based metabolomics. Multivariate analyses by partial least-squares projections to latent structures-discrimination analysis (PLS-DA) and PLS-regression offered extensive information for quality differentiation and quality evaluation, respectively. The impact of watermelon and rootstock cultivars on the sensory qualities of watermelon was determined on the basis of ^{1}H NMR metabolic fingerprinting and profiling. The significant metabolites contributing to the discrimination were also identified. A multivariate calibration model was successfully constructed by PLS-regression with extremely high reliability and accuracy. Thus, ^{1}H NMR-based metabolomics with multivariate analysis was considered to be one of the most suitable complementary techniques that could be applied to assess and predict the sensory quality of watermelons and other horticultural plants.

KEYWORDS: Metabolomics; ^{1}H NMR; PLS-regression; quality prediction; *Citrullus lanatus*; watermelon

INTRODUCTION

The demand for fresh-cut fruits and vegetables has been increasing due to the ease of intake and nutritional benefits due to the health-promoting effect of the synergistic combination of antioxidants, phytochemicals, and dietary fibers (*1, 2*).

Watermelon (*Citrullus lanatus* (Thunb.) Matsum. & Nakai) is an important fresh-cut fruit rich in vitamins A and C, fiber, and lycopene, an important carotenoid that provides the red color (*1, 3*). An intake of lycopene has several health benefits: it protects humans against certain types of cancer (*4*) and cardiovascular disease (*2, 5*) and leads to an enhancement of the immune system (*6*). Ever since the potential health benefits of fresh-cut fruits have become known, the consumption of fresh-cut fruits including watermelon has gradually started increasing. The market price of watermelon is directly dependent on its quality, which is generally assessed on the basis of the soluble solid content (SSC) contributed mostly by sugars (*7–11*).

The variations in the chemical constituents, especially SSC, have a significant impact on the quality of watermelon and are directly dependent on the storage temperature (*12*), cultivars (*13, 14*), and rootstock cultivars (*15–18*).

Grafting of vegetable plants is usually performed for controlling soil-borne diseases and nematodes, as well as for improving the tolerance of plants to environmental stresses (*19*). Grafting of watermelon tops on disease-resistant rootstocks such as gourd and squash is a common practice in Japan as it leads to better viability due to the increased resistance to plant fungus (*18, 20*); however, it has been reported that this practice results in the production of inferior-quality fruits (*16*).

Several spectroscopic and chromatographic techniques involving superconductive magnets, for example, magnetic resonance imaging (MRI) (*7, 21*), near-infrared reflectance (NIR) spectroscopy (*9, 22–24*), and high-performance liquid chromatography (HPLC) (*11*) have been widely applied either quantitatively or qualitatively to evaluate the quality of watermelons. However, several disadvantages such as the limitations of machine sensitivity and detectability and chemical specificity and solvent−eluent incompatibility limit the use of NIR and HPLC techniques in some cases. Recently, dielectric properties were also used to determine the sensing quality of watermelons. However, the quality of watermelons as evaluated from the dielectric properties and from the SSC was not as good as expected, and further studies are required to improve the prediction reliability (*10*).

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Table 1. Sample Identification and Quality Classification of 10 Diploid Watermelons[a]

samples	watermelon cultivar	rootstock cultivar	sensory quality		
			comprehensive	sweetness	crispness
777	Matsuribayashi 777	gourd (cv. Kachidoki 2gou)	1	4	1
HD	Harunodanran	gourd (cv. Kachidoki 2gou)	2	1	5
HW-TL45	HW-TL45	gourd (cv. Kachidoki 2gou)	4	3	2
ND	Natsunodanran	gourd (cv. Kachidoki 2gou)	5	5	3
FJ	Fujihikari	gourd (cv. Kachidoki 2gou)	8	8	7
MBNK	Matsuribayashi-NK	gourd (cv. Kachidoki 2gou)	9	9	10
ASR	Asahikari SR	gourd (cv. Kachidoki 2gou)	10	10	9
KG	Matsuribayashi 777	pumpkin (cv. Kagayaki)	3	2	4
V-999	Matsuribayashi 777	pumpkin (cv. V-999)	6	6	8
PST	Matsuribayashi 777	pumpkin (cv. Shintosa)	7	7	6

[a] Obtained from Hagiwara Farm Co. Ltd., Japan.

A high-throughput analytical technique such as nuclear magnetic resonance (NMR) spectrometry offers a wide range of information on metabolites and is considered to be the most suitable tool for both quality assessment and prediction due to its nonselective characteristic; in addition, this technique provides a large amount of structural information, simplifies sample preparation, and reduces the analysis time. This information, in combination with chemometrics by multivariate analysis, is expected to offer a conclusive idea of quality discrimination and prediction with high reliability and consistent results.

Although the quality of watermelons is mainly determined by the SSC, in practice, watermelon-producing companies consider not only the SSC but also other sensory properties such as the taste (sweet and sour), firmness, juiciness, flesh texture (whether crisp or not), flesh color, and fiber content of the fruit; these parameters are considered to be the comprehensive quality indices.

Here, we describe the effects of different cultivars of watermelons and rootstocks on the comprehensive sensory quality of the watermelons, which is its quality as determined from its appearance, taste, and flesh texture. A combination of nonselective ^{1}H NMR-based metabolomics and multivariate analysis by partial least-squares projections to latent structures-discrimination analysis (PLS-DA) and PLS-regression was established to obtain the quality discrimination and quantitative prediction model that could be applied to evaluate the sensory quality rankings of unidentified watermelon samples.

MATERIALS AND METHODS

Materials. Seven graded watermelons grafted on gourd (Kachidoki 2gou) rootstocks and three graded watermelons grafted on pumpkin (Shintosa, V-999, Kagayaki) rootstocks obtained from Hagiwara Farm Co. Ltd., Japan, were used in this study. The characteristics and sensory quality rankings of the watermelons were evaluated by the company's professional tasters, and these values are listed in **Table 1**. Three main characteristics, the comprehensive sensory quality, sweetness, and crispness, were selected to judge the quality of the watermelons.

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

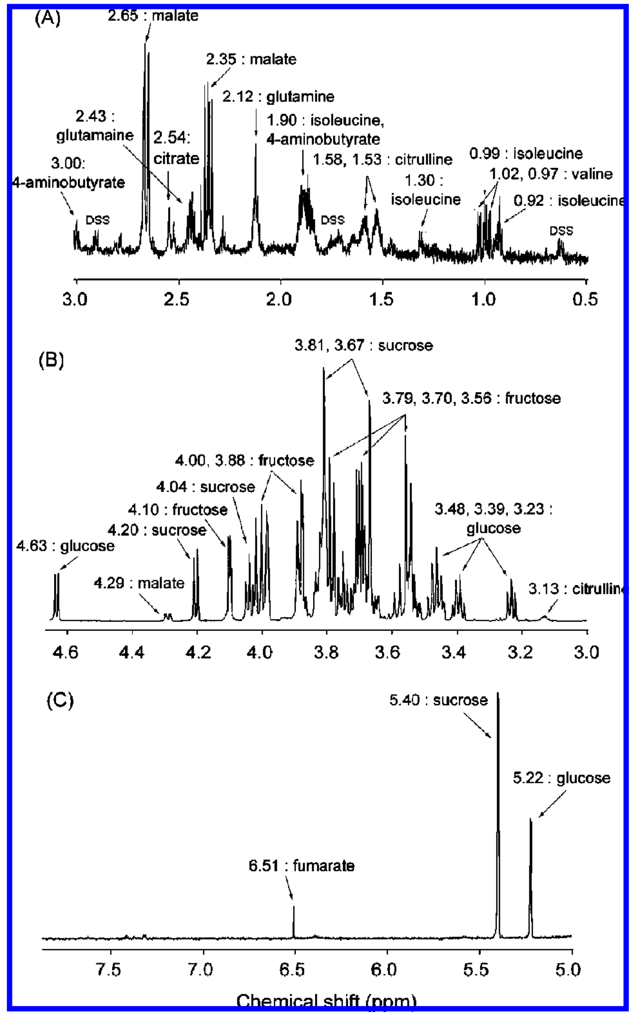

Figure 1. ^{1}H NMR spectra (750 MHz, D_2O) of the watermelon extracted from the highest quality sample in **(A)** high-, **(B)** middle-, and **(C)** low-frequency regions; the spectra were recorded at 25 °C.

Sample Preparation for ^{1}H NMR Analysis. Juice was extracted from the center and placenta of a watermelon separately and was mixed in a blender; then the juice was filtrated through a 0.45 μm PTFE membrane (Advantec, Dublin, CA). A 200 μL of supernatant juice was mixed with 200 μL of D_2O containing 3 mM DSS and 200 μL of 0.2 M phosphate buffer to make a 600 μL solution for NMR measurement. All samples were prepared in 1 day and kept at 4 °C prior to the analysis.

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 the internal standard at a chemical shift (δ) of 0.0 ppm, respectively. The ^{1}H NMR measurement was carried out with 64 transients and 128 K complex data points. With a 30° pulse angle, the acquisition time and recycle delay were 6.257 and 3.743 s per scan, respectively. The water suppression enhanced through T1 effects (WET) pulse sequence was applied to suppress the water signal. All spectra were Fourier transformed with 0.1 Hz line broadening prior to data reduction and preprocessing.

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 regions having an equal bin size

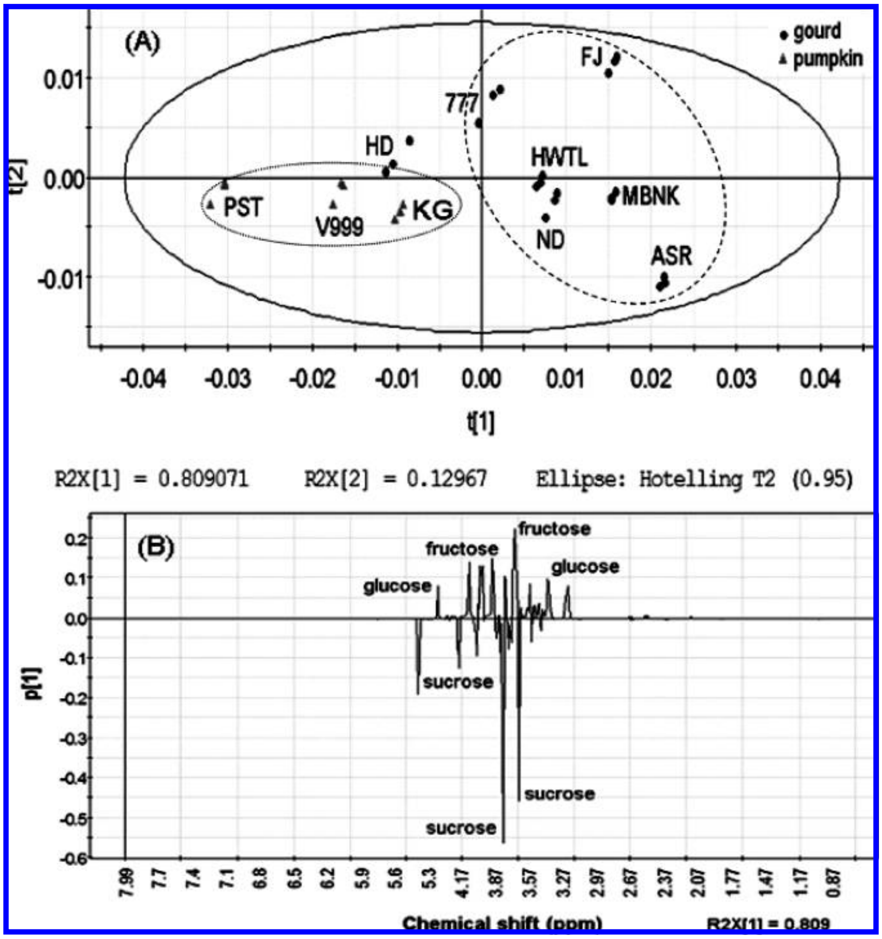

Figure 2. PLS-DA of NMR profiles of the central part of watermelons in the frequency region between δ 0.8 and 8.0 ppm, by excluding water region between δ 4.32 and 5.15 ppm. PLS-DA shows a clustering among watermelon cultivars grafted on gourd rootstock (♦) and pumpkin rootstock (▲). **(A)** PLS-DA score plot of the first and second PCs and **(B)** PLS-DA loading plot of the first component responsible for PLS-DA classification.

of 0.01 ppm over a range of δ 0.8 to 8.0 ppm, while the water resonance between δ 4.32 and 5.15 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 bins to the Microsoft Excel format (*.xls), which was then imported to SIMCA-P software, version 11 (Umetrics AB, Umeå, Sweden) for multivariate analysis.

^{1}H NMR Linear Regression Model. PLS regression was chosen to create a prediction model, in which the features from principal component analysis (PCA) and multiple regression were generalized and combined. This approach relates two data matrices, **X** and **Y**, by a linear multivariate model. This model tries to find the multidimensional direction in the **X** space that explains the maximum multidimensional variance direction in the **Y** space. The model is used to predict a dependent variables data set from a large set of independent variables with an ability to analyze data with noisy and incomplete variables in both matrices (*25, 26*).

In this study, PLS regression was carried out by using the SIMCA-P software, version 11; mean centering was used as the preprocessing method without any transformation.

RESULTS AND DISCUSSION

Identification of Chemical Constituents of Watermelon. The sensory quality of watermelons was evaluated by the company's professional tasters, and the values are listed in **Table 1**. In each quality category, No. 1 indicates the best quality, while No. 10 indicates the worst quality.

That the sensory quality of watermelon is dependent on its chemical constituents was identified from the best comprehensive quality watermelon juice (Matsuribayashi 777 grafted on gourd). The resulting ^{1}H NMR spectrum of the best quality watermelon is represented in **Figure 1**. The ^{1}H NMR resonances of hydrophilic metabolites were assigned by comparing them to the standard compounds in the Chenomx Suite 4.6, 800 MHz (pH 4−9) library database. The corresponding resonances were almost in good agreement with the standard signals. However, some resonances were slightly different from the values in the library database due to the difference in the measurement conditions.

About 11 major compounds were assignable, including isoleucine (δ 0.92, 0.99, 1.30, 1.90 ppm), valine (δ 0.97, 1.02 ppm), citrulline (δ 1.53, 1.58, 3.13 ppm), 4-aminobutyrate (δ 1.90, 2.28, 3.00 ppm), glutamine (δ 2.12, 2.43 ppm), malate (δ 2.35, 2.65, 4.29 ppm), citrate (δ 2.54 ppm), glucose (δ 3.23, 3.39, 3.48, 4.63, 5.22 ppm), fructose (δ 3.56, 3.70, 3.79, 3.88, 4.00, 4.10 ppm), sucrose (δ 3.67, 3.75, 3.81, 4.04, 4.20, 5.40 ppm), and fumalate (δ 6.51 ppm).

Metabolite Fingerprinting of Watermelon Metabolites: Effect of Grafting Method and Cultivar on Chemical Constituents Related to Sensory Quality Evaluation. Multivariate analysis was applied to ^{1}H NMR data by sorting the spectra data sets into categories, where the complicated signal assignments were left behind (*27*). In this study, PLS-DA was employed as the supervised pattern-recognition method to analyze the ^{1}H NMR spectra data in a frequency region between δ 0.80 and 8.00 ppm, with an exclusion of water resonance at δ 4.32−5.15 ppm. In the case of high-quality watermelon, the sensory characteristics are theoretically identical for the entire

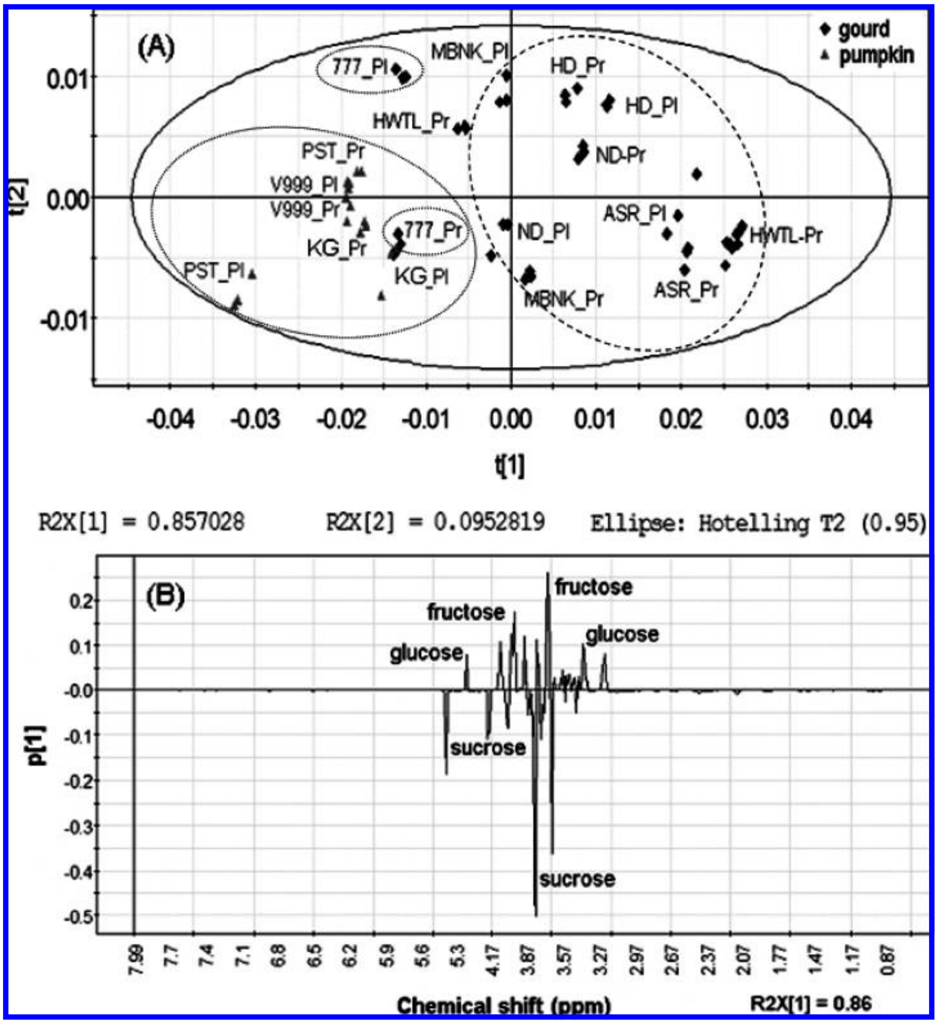

Figure 3. PLS-DA of NMR profiles of the placenta (left and right) parts of watermelons in the frequency region between δ 0.8 and 8.0 ppm, by excluding water region between δ 4.32 and 5.15 ppm. PLS-DA shows a clustering among watermelon cultivars grafted on gourd rootstock (♦) and pumpkin rootstock (▲). **(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.

fruit. However, in general, some sensory characters such as sweetness and crispness vary depending on the position of the flesh. Thus, it is necessary to investigate the sensory quality of discrete portions independently, i.e., the flesh corresponding to the central and placenta parts should be investigated separately. The resulting PLS-DA plots of the central and placenta parts of watermelon are shown in **Figures 2** and 3, respectively. Seven watermelon cultivars, Matsuribayashi 777 (777), Harunodanran (HD), HW-TL45 (HW-TL45), Natsunodanran (ND), Fujihikari (FJ), Matsuribayashi-NK (MBNK), and Asahikari (ASR), that were grafted on gourd rootstock (cv. Kachidoki 2gou) and one cultivar, 777, grafted on three different pumpkin rootstocks (cv. Shintosa (PST), V-999, and Kagayaki (KG)) were employed in this study. It should be noted that only Matsuribayashi 777 was grafted on both gourd and pumpkin rootstocks to study the effect of rootstock cultivars in quality discrimination.

In the PLS-DA score plot of the central part of different watermelons shown in **Figure 2**A, a clear clustering was observed in relation to their rootstock cultivars in the first and second principal components (PC1/PC2). Except for HD, all watermelons grafted on gourd were differentiated from those grafted on pumpkin along the PC1 axis having a positive correlation. It has been reported that plant cultivar is one of the major factors influencing the metabolites and quality of fruits (*13, 14*). To investigate the effect of plant cultivar on the chemical constituents related to sensory quality, a similar watermelon cultivar was employed. It should be noted that in the PLS-DA result, the watermelon cultivar Matsuribayashi 777 was clustered into two categories in relation to the rootstock cultivars: the ones grafted on pumpkin rootstocks (KG, V-999, and PST) were differentiated from those grafted on gourd (777). The corresponding loading plot shown in **Figure 2**B indicates that the constituents of watermelon grafted on gourd were mainly reducing sugars such as glucose and fructose. On the other hand, the watermelon cultivars grafted on pumpkin were rich in sucrose, a nonreducing sugar. These results demonstrate that the quantity of sugar metabolites in the central flesh tissues is largely dependent on the rootstock cultivar; the quantity of sugar metabolites is in turn responsible for the sensory quality. On the contrary, the watermelon cultivar had no significant effect on the properties of the flesh tissues from the central part of the watermelon. The effect of the rootstock cultivar on the chemical constituents and quality of watermelon could be observed through a change in the sugar metabolism related to the enzyme activity (*16*) and the differences in the root cytokinin activity due to the assimilate supply translocated from the shoot (*15*). It should be noted that HD contained distinctive constituents similar to watermelons grafted on pumpkin, namely, a higher amount of sucrose; however, it differed from those grafted on gourd (**Figure 2**). According to

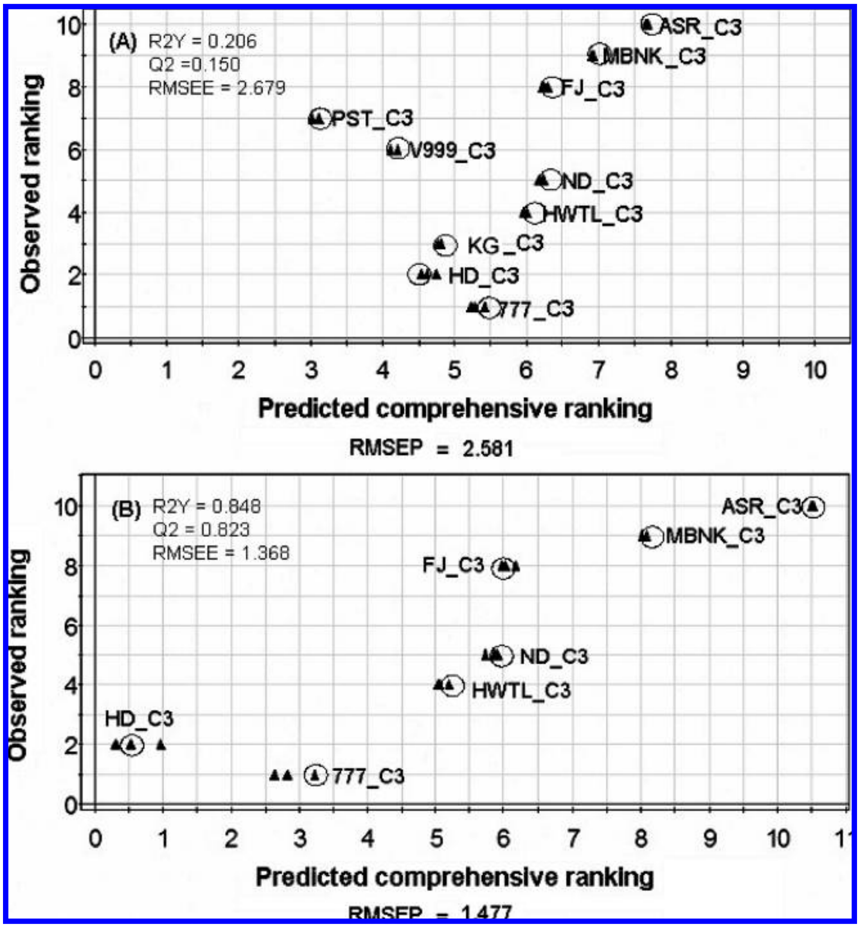

Figure 4. Relationship between observed and predicted comprehensive quality ranking of the central part of watermelon obtained from the PLS-regression model with validation set. The comprehensive sensory quality is implemented as a response variable index. The prediction model is calculated from ^{1}H NMR data in a region of δ 0.8−8.0 ppm by excluding the water region between δ 4.32 and 5.15 ppm: **(A)** regression model of watermelon cultivars grafted on gourd and pumpkin rootstocks and **(B)** regression model of watermelon cultivars grafted on gourd rootstock.

the sensory sweetness rankings provided in **Table 1**, HD and KG were the sweetest and second sweetest watermelons, respectively. Based on this sensory evaluation, it could be inferred that sucrose was the predominant biochemical component contributing to the sweetness of watermelon, considered by company veterans for scoring the sensory sweetness quality. This is in good agreement with a previous report showing that the types and amount of sugars had a direct influence on the flavor and quality of fruits (*28*). The perceived sweetness value of fructose is high at about 1.40−1.75, while those of sucrose and glucose are 1.0 and 0.60−0.75, respectively (*29*); however, the total average sucrose content of HD and all watermelons grafted on pumpkin was higher than that of 777. With regard to this result, it could be confirmed that sucrose was the significant sugar metabolite influencing the sensory sweetness quality, as stated previously. It should be noted that V-999 (Matsuribayashi 777 grafted on pumpkin cv. V-999) and PST (Matsuribayashi 777 grafted on pumpkin cv. Shintosa) were sixth and seventh on the sweetness scale, respectively; however, they were clustered in the same category as HD and KG, signifying an inconsistent and low accuracy of the sensory evaluation result. Consequently, a new sensory quality prediction methodology should be developed to improve the prediction accuracy and enhance its reliability.

The PLS-DA score and loading plots of the left (Pl) and right (Pr) parts of the placentas of different watermelons are illustrated in **Figure 3**A and B, respectively. The score plot displayed in **Figure 3**A shows a clustering of watermelons in the first and second PCs in relation to their rootstock or watermelon cultivars. The former potential effect, however, could be neglected by considering the fact that the PLS-DA separated the watermelons grafted on similar gourd rootstock into two categories, where 777 (Pl and Pr) had a negative correlation with PC1. On the contrary, the cultivars of the other six watermelons exhibited a positive relationship. This indicated clearly that the rootstock cultivar had no significant effect on a clustering. It is noteworthy that all Matsuribayashi 777 cultivars, including the ones grafted on gourd (777) and the ones grafted on pumpkin (KG, V-999, and PST), were clearly differentiated from the other cultivars on the PC1 axis having a negative correlation. The corresponding loading plot (**Figure 3**B) illustrated that the increase in the sucrose concentration contributed most to the separation of Matsuribayashi 777 from the cultivars of the other watermelons. Fructose and glucose, therefore, were the significant reducing sugars that were present mainly in the placenta of the other cultivars. The above results demonstrate that the chemical constituents in the placenta are strongly affected only by the watermelon cultivars, whereas the rootstock cultivars did not have a significant impact on the changes in the metabolites. These recent findings, however, are contradictory to those obtained from the analyses of the central flesh tissue. The rootstock cultivar had a significant effect on the changes in the metabolites only in the central fleshy part; on the other hand, the watermelon cultivar was a foremost factor contributing to the metabolite changes in the placenta.

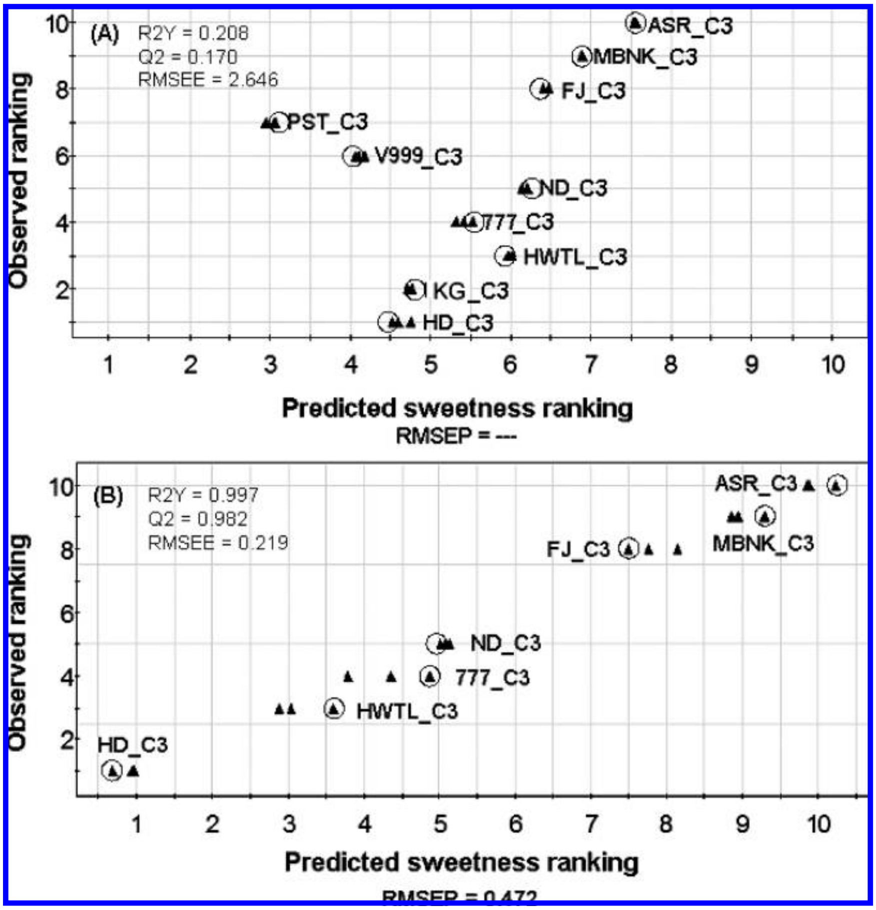

Figure 5. Relationship between observed and predicted quality ranking of the central part of watermelon obtained from the PLS-regression model with validation set. The sweetness character is implemented as a response variable index. The prediction model is calculated from ^{1}H NMR data in a region of δ 0.8−8.0 ppm, by excluding water region between δ 4.32 and 5.15 ppm: **(A)** regression model of watermelon cultivars grafted on gourd and pumpkin rootstocks and **(B)** regression model of watermelon cultivars grafted on gourd rootstock.

As described elsewhere, some sensory characteristics, such as sweetness, are generally inconsistent in the entire fruit due to variations in the sugar constituents and their concentrations. On the basis of our recent results, the dissimilarities between the characteristics of the central flesh tissue and the placenta flesh tissue were attributed to the differences in the sugar metabolism controlled individually by the cultivars of the rootstock and watermelons.

Sensory Quality-Predictive Model by PLS Regression: Effect of Rootstock Cultivars on the Model's Predictive Ability for Central Flesh Tissues. As indicated previously, inconsistent, low-accuracy quality evaluation results were obtained in the case of the sensory evaluation method; further, the evaluation was a time-consuming process. As to these drawbacks, a combination technique between nonselective ^{1}H NMR data and chemometric by PLS was introduced to offer the high reliability and high accuracy quality predictive model respective of its sensory evaluation score via a regression model. The prediction model is first proposed based on the system behavior, followed by a calibration step, where the optimal values for model parameters respected to training samples are determined. Finally, the values of unknown independent variables were predicted in the prediction step by using the resulting training model (*30*). In this study, PLS regression was implemented to predict the sensory quality of the central and placenta flesh tissues of watermelons and validate the most applicable sensory trait. Three main sensory quality characteristics, comprehensive sensory quality, sweetness, and crispness, were applied individually as a response index, while the model predictability and prediction accuracy were verified accordingly. The entire data set was divided into two parts, training and validation, which were used to create a prediction model and to verify the model's predictability, respectively. A correlation coefficient, R^2, a cross-validated correlation coefficient, Q^2, and the validation error given as the root-mean-square error of prediction, RMSEP, are generally used to verify the regression model quality. R^2 is used to describe how well the data of the training set, which can vary between 0 and 1 (where 1 implies a perfectly fitting model), can be mathematically reproduced. The Q^2 value can be used to determine how good a prediction model is. When $Q^2 > 0.5$, the prediction model is considered to have good prediction ability, and if $Q^2 > 0.9$, it is considered to have excellent predictive ability (*31*).

The PLS relationship between the measured and the predicted comprehensive sensory quality ranking of the central part of the watermelons grafted on both gourd and pumpkin rootstocks is illustrated in **Figure 4**A. The duplicated ^{1}H NMR spectra of each cultivar were used to construct the regression model, while the 3-series watermelon samples were used as the validated samples for model validation. The data was preprocessed by mean centering before analysis, without any scaling and transformation. The PLS regression results were as follows: R^2, which gives the accuracy of prediction, was 0.206; Q^2, which gives the predictability of the model, was 0.150; and RMSEP was 2.581. The extremely low R^2 and Q^2 values and the large RMSEP value clearly indicated the inefficiency of regression fitting and predictability of the resulting PLS model. In addition,

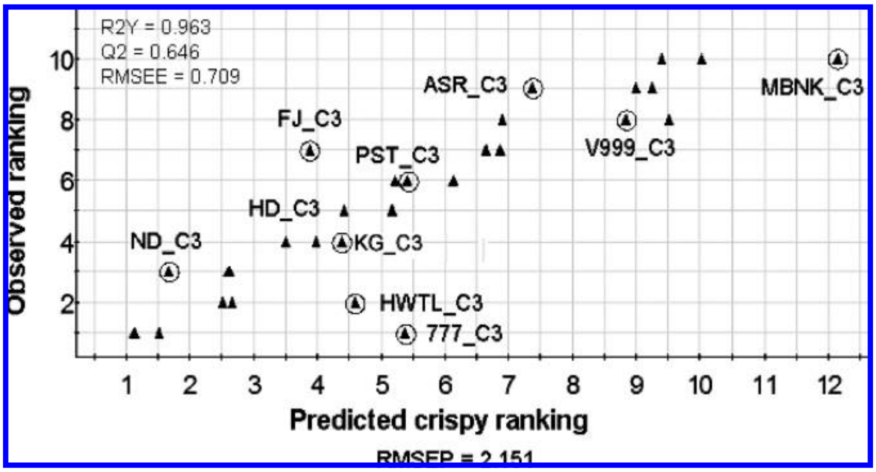

Figure 6. Relationship between observed and predicted quality ranking of the central part of watermelon cultivars grafted on gourd and pumpkin rootstocks obtained from the PLS-regression model with validation set. The crispy character is implemented as a response variable index. The prediction model is calculated from ^{1}H NMR data in a region of δ 0.8−8.0 ppm, by excluding water region between δ 4.32 and 5.15 ppm.

it was apparent from this model that PST and V-999, the watermelon cultivars grafted on pumpkin rootstock, were scattered from a diagonal regression line. It was thereby concluded that the rootstock cultivar had a strong impact on the quality and predictability of the prediction model when the comprehensive sensory ranking was employed as a response index.

The predictive ability of the model could be improved only when the watermelons grafted with a particular rootstock were utilized. The PLS regression of the watermelons grafted only on gourd rootstock is shown in **Figure 4**B. By excluding the ^{1}H NMR data sets of the watermelons grafted on pumpkin rootstocks, the accuracy and predictability of the model increased drastically, as observed from the increase in the values of R^2 and Q^2 from 0.206 and 0.150 to 0.848 and 0.823, respectively. An improvement in the predictive model's accuracy and predictability after the elimination of rootstock-dependent variables is a strong indicator of the fact that the rootstock cultivar has a large impact on the comprehensive sensory prediction model. Hence, the resulting model could predict precisely only in the case of watermelons grafted on a particular rootstock, which reveals that the model has an unreliable prediction ability.

Among the sensory quality evaluation parameters, the sweetness is one of the most important parameters taken into consideration by professional watermelon tasters. The sensory sweetness quality was then used to construct a quality prediction model. The resulting PLS regression model is shown in **Figure 5**A. The result is similar to that of the comprehensive quality ranking prediction. As observed in this figure, PST and V-999 are out of a diagonal regression line. The corresponding R^2 and Q^2 values of this model were very low, about 0.208 and 0.170, respectively. Furthermore, the fact that the RMSEP value could not be determined indicates that a large prediction error was obtained. As expected, the accuracy and predictability of the model improved significantly when the rootstock-dependent variables, that is, the watermelons grafted on pumpkin rootstocks, were eliminated. An enhancement of the model predictability can be clearly observed in **Figure 5**B. The R^2 and Q^2 values increased drastically from 0.208 and 0.170 to 0.997 and 0.982, respectively, with an RMSEP value of 0.472. This indicates that the grafting method had an impact on the sensory sweetness quality evaluation model.

As mentioned previously, the sensory sweetness and comprehensive sensory quality are largely influenced by the amounts of sugars, either reducing or nonreducing. The sugar content in fruit depends on the rootstock cultivar, as described in the discrimination results; accordingly, the differences in the quality prediction model obtained by PLS-regression were attributed to the significant differences in the types and quantities of sugar.

In general, the sensory quality of watermelon was judged not only from its taste or appearance but also from other physical properties such as its crispness. Consequently, the crispness was also considered in the construction of the quality predictive model. The correlation between the observed and predicted crispiness quality ranking of the central flesh tissues is shown in **Figure 6**. The corresponding R^2 and Q^2 values are 0.963 and 0.646, respectively, demonstrating a high accuracy and moderate predictability of the resulting regression model. Unlike the models in which the comprehensive sensory quality and sweetness were considered, a suitable prediction model was obtained without any exclusion of rootstock-dependent variables when the crispness was assessed. Crispness is a physical characteristic of the flesh texture; unlike the comprehensive sensory quality and sweetness, the crispness is not directly related to the sugar content in the fruit. The R^2 and Q^2 values of this model were relatively high; however, when the regression predictability was verified by the C3 series validation set, the corresponding validation error was quite large, with an RMSEP of 2.151. In addition, the predicted ranking of the validation samples was slightly scattered from the diagonal regression line, signifying a small distortion of the prediction model. These results reveal that the crispness can be used to construct a quality prediction model of central flesh tissues of watermelons but is not the most suitable sensory character, as it leads to a small model distortion.

Sensory Quality-Predictive Model by PLS Regression: Effect of Rootstock Cultivar on the Model Predictive Ability for Placenta Flesh Tissues. As indicated elsewhere, the inconsistency in the quality of the watermelon in the entire fruit is due to the differences in the constituents and their content; this inconsistency is expected to play an important role in the quality of the subsequent prediction model. Thus, it is important to compare the prediction results obtained from the central flesh tissues to those acquired from the placenta using the similar response variables. Accordingly, the best prediction model can be determined.

Figure 7A−C show the PLS quality prediction models when the comprehensive sensory quality, sweetness, and crispness were applied as response variables in the prediction model of

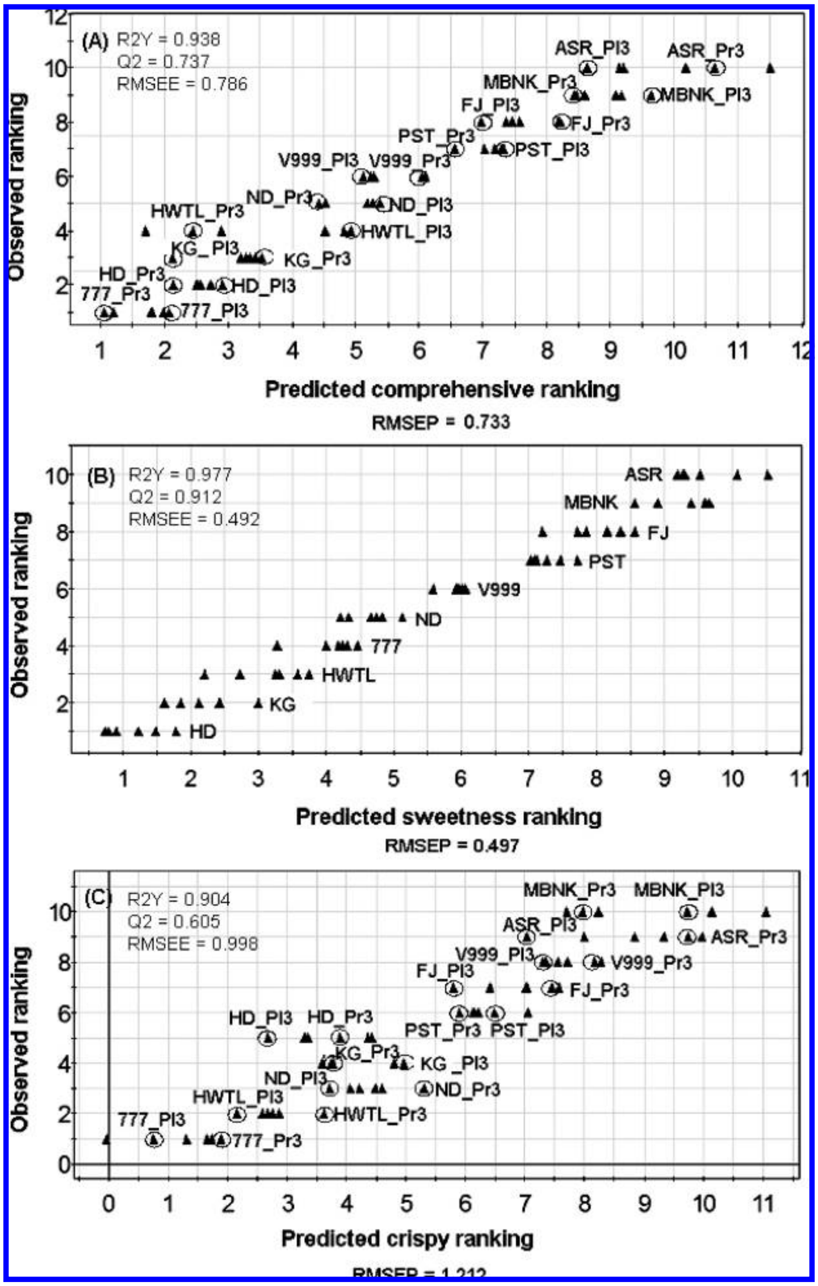

Figure 7. Relationship between observed and predicted sensory quality of the placenta parts of watermelon obtained from the PLS-regression model with validation set. The prediction model is calculated from ^{1}H NMR data in a region of δ 0.8–8.0 ppm, by excluding the water region between δ 4.32 and 5.15 ppm, using **(A)** comprehensive, **(B)** sweetness, and **(C)** crispy characters as response variable indices.

placenta flesh tissues, respectively. A distortion in the model due to the rootstock cultivar was not observed in the results of the regression models of the placenta flesh tissue, unlike the case of the model of the central fleshy part. All placenta samples were well aligned along the regression line without large scattering. These results indicate that the rootstock cultivar did not have a significant influence on the quality of the prediction model of placenta fleshy tissues.

All prediction models yielded very high R^2 values of more than 0.9, thereby demonstrating excellent fitting and extremely high accuracy. In addition, the Q^2 values varied from 0.6 to greater than 0.9, indicating that the model predictability varied from fair to very high. The quality of the placenta flesh tissues could then be predicted accurately in relation to their comprehensive sensory quality, sweetness, and crispness. The best prediction model with excellent accuracy, highest predictability, and lowest validation error was achieved when the sweetness was predicted as having very high R^2 and Q^2 values of 0.977 and 0.912, respectively, with a small RMSEP of 0.497. By considering that sugar compounds play an important role in the sweetness, the types and quantity of the sugar compounds could be related to the quality of the sweetness prediction model, providing the best and most important prediction results.

In summary, ^{1}H NMR spectrometry provided useful information about the relationship between the principal metabolites and the sensory characteristics. A combination of this technique with multivariate analysis had several advantages over ordinary sensory quality evaluation methods in the cases of both qualitative as well as quantitative analysis. PLS-DA results revealed the effect of the types and amounts of sugar on the sensory quality of the watermelon cultivars and their rootstock cultivars. On the basis of the discrimination results, it was postulated that sucrose was the most significant metabolite affecting the sensory quality evaluation. Multivariate analysis by PLS-regression revealed a strong impact of sugar compounds in the rootstock cultivars on the quality of the sensory prediction model. However, when the effects of the rootstock cultivar were reduced, the results of this model in terms of the comprehensive, sweetness, and crispness sensory quality predictions were still highly reliable and accurate. Thus, for evaluating the quality of watermelons, metabolomics is expected to be one of the best methodologies for both qualitative and quantitative evaluation.

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