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Gas Chromatograph Mass Spectrometer

Construction of a Regression Model for a Coffee Sensory Evaluation Through the Comprehensive Analysis of Metabolites

Taste evaluation of food is an extremely important factor for food product development and quality evaluation. While there is a relationship between the taste of a food item and its components to a certain extent, simple one-to-one relationships between a component and a taste are rare, with generally multiple components complexly affecting the taste. In some cases, this makes it difficult to improve and evaluate the quality of a food product based on component measurement.

Against this backdrop, in recent years the food and other industries are increasingly showing interest in performing regression analysis on the results of sensory evaluations through the comprehensive analysis of metabolites. One comprehensive analysis method which is expected to become an extremely useful tool for such analyses is widely targeted metabolomics. Enabling favorable peak identification, it allows examination of components that contribute to taste after the construction of a model from sensory evaluation results.

Eight types of coffee beans were ground, roasted, and extracted under the same conditions and subjected to sensory evaluation. Metabolites were then extracted from each coffee bean type and measured using GC-MS/MS analysis. Using the results of the sensory evaluation as response variables and the processed values of the detected peak areas of metabolites as explanatory variables, a partial least squares (PLS) regression model of the relationship between these variables was constructed. The components that affect the taste were then examined using the results of the PLS regression model.

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Sensory Evaluation

Using a five-point rating scale, eight panelists rated the bitterness of eight types of coffee (A through H) that were ground, roasted, and extracted under the same conditions. The scores were summed to create a total score. The results are shown in Table 1.

Table 1 Results of Sensory Evaluation

	A	B	C	D	E	F	G	H
Bitterness Score	34	28	25	19	20	22	25	22

Analysis of Metabolites in Coffee Beans

Coffee beans that were ground and roasted under the same conditions were pre-treated as described in Fig. 1 and each sample was analyzed three times by GC-MS/MS analysis. Analysis conditions were based on the Smart Metabolites Database (Table 2).

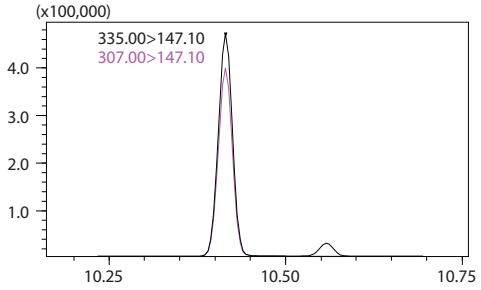
Table 2 Analytical Conditions (Based on the Smart Metabolites Database)

Column	: BPX-5 (30 m × 0.25 mm, 0.25 µm)
Injection mode	: Split
Split ratio	: 30:1
Injection port temperature	: 250 °C
Oven temperature program	: 60 °C → (15 °C/min) → 330 °C (3 min)
Flow control	: Linear velocity (39.0 cm/sec)
Purge flow rate	: 5 mL/sec
Interface temperature	: 200 °C
Ion source temperature	: 280 °C
Event time	: 0.25 sec

1. Weigh out approx. 20 mg of coffee beans into a 1.5-mL tube.
2. Add 10 µL of 2-Isopropylmalic acid aqueous solution (20 mg/mL).
3. Add 500 µL of methanol.
4. Add 250 µL of ultra-pure water and mix well.
5. Transfer 600 µL of the suspension into a new 1.5-mL tube.
6. Add 400 µL of chloroform and mix well.
7. Centrifuge for three minutes at 16,000 G and collect 200 µL of the supernatant.
8. Dewater the supernatant in a centrifugal concentrator over one night to completely dry it.
9. Dissolve methylamine hydrochloride in pyridine to create a 20 mg/mL solution.
10. Add 80 µL of the solution created in step 9 to the dried tube.
11. After completely dispersing and dissolving the residue in the solution using a sonicator, shake for 90 minutes at 30 °C.
12. Add 40 µL of N-Methyl-N-(trimethylsilyl) trifluoroacetamide and shake for 30 minutes at 37 ° C.
13. Place in a GC-MS vial and analyze.

Fig. 1 Method for Extracting Metabolites from Coffee Beans

Among the 475 components measured using the Smart Metabolites Database, 192 components were detected in all eight samples. The area value of these 192 components was divided by the peak area value of the internal standard and then normalized to have a mean of 0 and a deviation of 1. These normalized values were used as the explanatory variables data set.



ID	Compound / Sample	A_01	B_01	C_01	D_01	E_01
	Bitterness	34	28	25	19	2
52	2-Propyl-5-hydroxy-pentanoic acid-2	0.030737	0.023865	0.024535	0.015942	0.0238
53	Threitol-4TMS	0.574396	0.556411	0.654507	0.478847	0.1364
54	Dihydrouracil-TMS	0.055576	0.056699	0.04577	0.037841	0.04076
55	Malic acid-3TMS	11.62529	11.05202	9.711597	8.162754	8.58277
56	meso-Erythritol-4TMS	1.433843	1.326945	1.185678	1.0577	1.06618
57	Niacinamide-TMS	0.020438	0.017427	0.022618	0.036423	0.03219
58	N-Acetylserine-2TMS	0.12085	0.127045	0.109326	0.126517	0.10657
59	Aspartic acid-3TMS	0.066144	0.134722	0.052077	0.125087	0.14311
60	3-Aminoglutaric acid-2TMS	0.075891	0.144157	0.066347	0.134849	0.05790
61	4-Hydroxyproline-3TMS	0.024839	0.018295	0.030287	0.021463	2.71109
62	4-Aminobutyric acid-3TMS	0.202442	0.115015	0.048878	0.143864	0.22620
63	5-Oxoproline-2TMS	79.23318	83.43766	73.40937	78.22139	90.1037
64	Cytosine-2TMS	0.165294	0.186654	0.133193	0.179156	0.12290
65	Threonic acid-4TMS	0.600645	0.404187	0.538817	0.423448	0.50393

Fig. 2 Example Analysis (Chromatogram of Malic acid-3TMS and a Partial Data Set)

Construction of a PLS Regression Model

Using the data obtained through sensory evaluation as response variables and the data obtained via GC-MS/MS measurement as explanatory variables, a regression model was created for regressing response variables on the explanatory variables by the PLS method. The relationship between the regressed (predicted) and the actual response variables is shown in Fig. 3. The SIMCA multivariate analysis software 14 was used to perform the PLS regression.

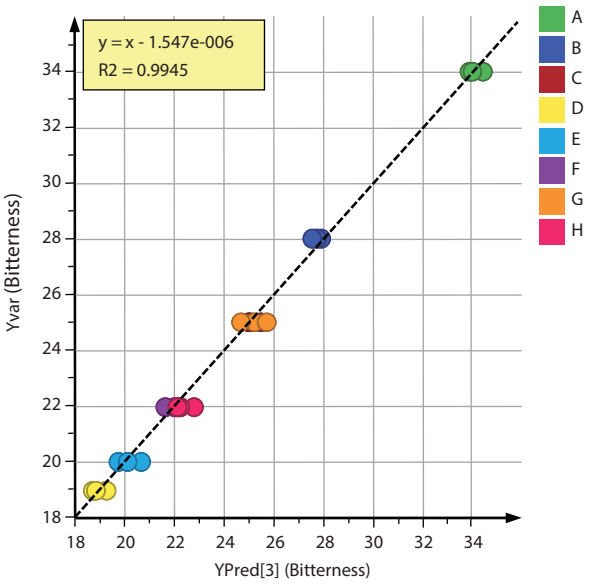


Fig. 3 Bitterness Score Prediction Plot

The actual bitterness scores are on the vertical axis. The horizontal axis indicates the bitterness scores predicted from the behavior of the area values of metabolites based on the model equation. The root mean square error of prediction (RMSEP), which indicates the mean deviation from the prediction model equation, was 0.346 and the correlation coefficient was 0.9945.

Tables 3 and 4 show the regression coefficients and the variable importance in projection (VIP) scores from this model.

Table 3 Compounds with a Large Regression Coefficient (Positive) and Their VIP Score

Compound Name	Regression Coefficient	VIP
Glycine-3TMS	0.047	1.648
Arabitol-5TMS	0.043	1.682
Mannitol-6TMS	0.042	1.783
Glucose-meto-5TMS (2)	0.041	1.772
3-Phenyllactic acid-2TMS	0.037	1.591
Lauric acid-TMS	0.036	1.245
Glucuronic acid-meto-5TMS (2)	0.035	1.555
Octanoic acid-TMS	0.034	1.047
2-Aminoethanol-3TMS	0.034	1.417

Table 4 Compounds with a Large Regression Coefficient (Negative) and Their VIP Score

Compound Name	Regression Coefficient	VIP
4-Hydroxybenzoic acid-2TMS	–0.037	1.574
Glyceraldehyde-meto-2TMS (2)	–0.037	1.578
Erythrulose-meto-3TMS (2)	–0.034	1.383
Gluconic acid-6TMS	–0.033	1.342
Coniferyl aldehyde-meto-TMS (2)	–0.033	1.332
5-Oxoproline-2TMS	–0.032	1.068
Niacinamide-TMS	–0.031	1.505
Dihydroxyacetone-meto-2TMS	–0.031	1.215
Tryptamine-2TMS	–0.031	1.275

All components having a regression coefficient with a large absolute value had VIP scores greater than 1, indicating their importance in regression. Also, the absolute values of regression coefficients indicate that samples with large peaks of Glycine-3TMS, Arabitol-5TMS, Mannitol-6TMS, etc., have high bitterness scores while samples with large peaks of 4-Hydroxybenzoic acid-2TMS, Glyceraldehyde-meto-2TMS(2), Erythrulose-meto-3TMS(2), etc., have low bitterness scores.

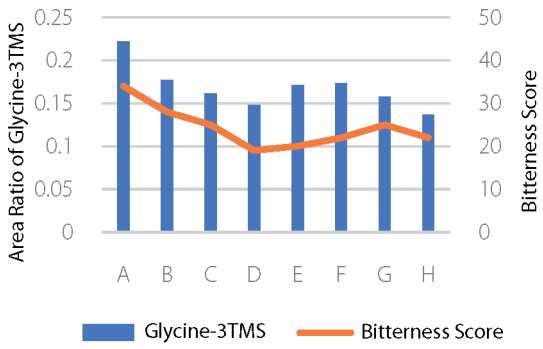


Fig. 4 Area Ratio of Glycine-3TMS

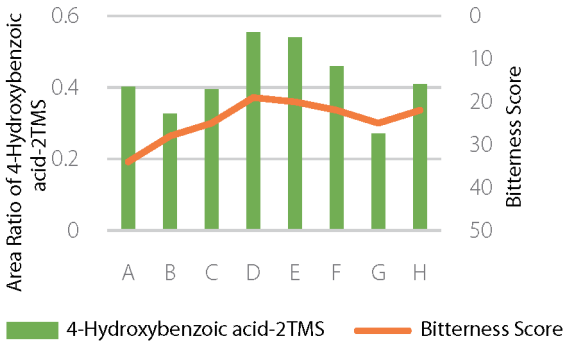


Fig. 5 Area Ratio of 4-Hydroxybenzoic acid-2TMS

* The panelists involved in the sensory evaluation described in this issue of Application News are not trained panelists.

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