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Application News

Comparison of Primary and Secondary Metabolite in Ginger Cultivars

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User Benefits

- Both hydrophilic and hydrophobic metabolites can be analyzed serially using the same column.
- The Multi-Omics Analysis Package can be used for integrated analysis of hydrophilic and hydrophobic metabolites and for visualizing differences in metabolic pathways.

■ Introduction

Plants are known to generate secondary metabolites, such as terpenes and flavonoids, which help the body's defenses. They have also been widely used as flavors or functional ingredients. Ginger (*Zingiber officinale*) is a perennial plant that has a variety of uses. In Japan, different cultivars are used for different purposes. For example, ginger plants with large roots, such as Tosa ginger, are mainly used for culinary purposes, whereas those with small roots, such as Kintoki ginger, are used for medicinal purposes. These differences in their uses are thought to correlate with primary metabolites, which are associated with flavor and nutrition, and secondary metabolites, which have medicinal benefits. Therefore, in this Application News article, an integrative comparison of the metabolic profiles of primary metabolites (with a focus on hydrophilic metabolites) and hydrophobic secondary metabolites was used to clarify the differences between Kintoki and Tosa ginger cultivars.

Fig. 1 Kintoki Ginger (Left) and Tosa Ginger (Right)

■ Pretreatment and Analysis Conditions

Metabolomic analysis is typically used for hydrophilic metabolites. Even for GC-MS analysis, techniques that involve extracting the hydrophilic fraction, freezing it with nitrogen, and then treating it with methoxime-TMS (trimethylsilyl) derivatization is widely used. However, because most secondary metabolites are hydrophobic, the hydrophobic fractions must be fractionated separately. Therefore, for this article, a variation of the Bligh and Dyer method was used to extract both hydrophilic and hydrophobic metabolite fractions from a single sample, and 20 mg of frozen, ground, and then dried ginger was weighed out for use.

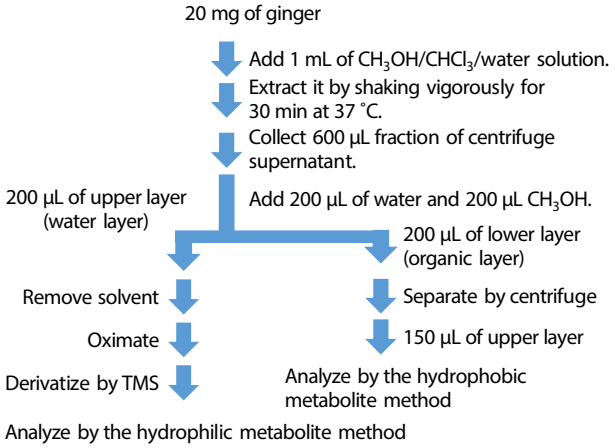

Fig. 2 Process Flow of Fraction Extraction

■ Analysis Conditions

Samples were analyzed using the following analytical conditions. The analysis conditions for the hydrophobic fraction that contained a large number of secondary metabolites are shown in Table 1, and the analysis conditions for hydrophilic metabolites are shown in Table 2.

Table 1 Analysis Conditions for Hydrophobic Products of Metabolism

System Configuration	
GC-MS:	GCMS-TQ™8040 NX
Column:	DB-5 (30 m × 0.25 mm I.D., df = 1.00 µm)
[GC]	
Injection Temp.:	250 °C
Injection Volume:	1 µL
Injection Method:	Split
Split Ratio:	5
Carrier Gas:	He
Control Method:	Constant linear velocity (42.5 cm/sec)
Column Oven Temp.:	60 °C (2 min) → (10 °C/min) → 320 °C (10 min)
[MS]	
Interface Temp.:	250 °C
Ion Source Temp.:	200 °C
Ionization Mode:	EI
Data Acquisition Mode:	Scan (*m/z* 45 to 500)

Table 2 Analysis Conditions for Hydrophilic Products of Metabolism

System Configuration	
GC-MS:	GCMS-TQ8040 NX
Column:	DB-5 (30 m × 0.25 mm I.D., df = 1.00 µm)
[GC]	
Injection Temp.:	250 °C
Injection Volume:	1 µL
Injection Method:	Splitless
Carrier Gas:	He
Control Method:	Constant linear velocity (42.5 cm/sec)
Oven Temp.:	100 °C (4 min) → (10 °C/min) → 320 °C (11 min)
[MS]	
Interface Temp.:	250 °C
Ion Source Temp.:	200 °C
Ionization Mode:	EI
Data Acquisition Mode:	MRM (using method in Smart Metabolites Database Ver. 2)

■ Analysis of Hydrophobic Products of Metabolism

The scan mode was used to analyze the hydrophobic metabolites. More peaks were detected with Kintoki than with Tosa ginger (Fig. 3), and the library search results indicated it contained many secondary metabolites were , such as phenols and terpenoids (Fig. 4). In addition, using a standard sample of 98 components, including compounds isolated from ginger, to identify compounds in the samples found that Kintoki ginger contains phenols, such as zingerone, and terpenoids such as aframodial and galanolactone. These secondary metabolites are reported to have a variety of medicinal benefits. So this provides strong support for Kintoki ginger being used for medicinal purposes.

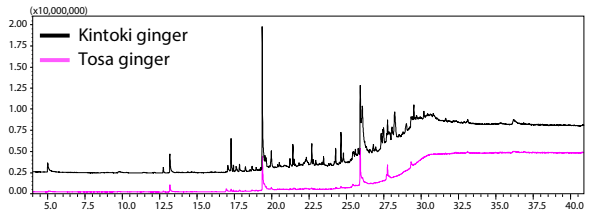

Fig. 3 Comparison of TIC Chromatograms for Kintoki and Tosa Gingers

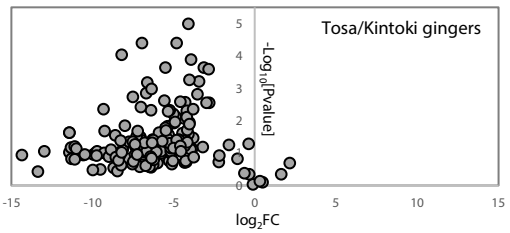

Fig. 4 Volcano Plot of Hydrophobic Metabolite Data Analysis Results

■ Analysis of Hydrophilic Products of Metabolism

Hydrophilic metabolites were analyzed using the Smart Metabolites Database. Principal component analysis (PCA) found that the Kintoki and Tosa ginger samples had differences in their hydrophilic metabolites. To discover the significance of the differences, the data were analyzed using a volcano plot (Fig. 5). This found that the Tosa ginger sample tended to have more primary metabolites and, in particular, more amino acids. This analysis of hydrophilic metabolites suggests that Tosa ginger is more suitable for culinary use than Kintoki ginger.

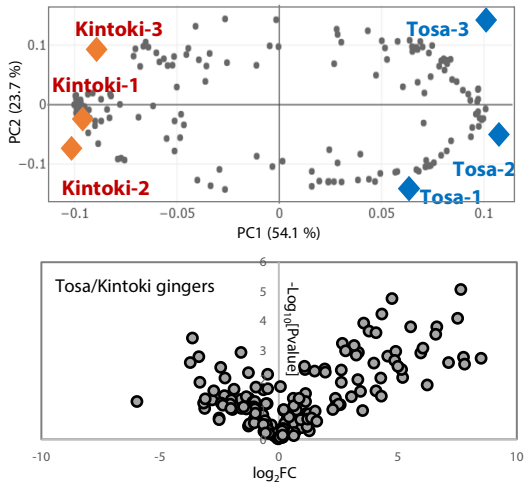

Fig. 5 PCA Results (Upper) and Volcano Plot (Lower) of Hydrophilic Metabolite Data

■ Metabolic Mapping Analysis

Scan analysis and PCA analysis found that the Kintoki ginger sample contained large numbers of secondary metabolites, whereas the Tosa ginger sample contained large numbers of primary metabolites, such as amino acids. Given that secondary metabolites are synthesized from primary metabolites, the cinnamic acid/monolignol pathway was drawn using the Multi-Omics Analysis Package (Fig. 6) to understand that relationship new cinnamic acid/monolignol pathways were discovered. These pathways are important for synthesizing secondary metabolites, such as phenols, lignins, and flavonoids. This analysis shows that Kintoki ginger tends to contain large amounts of upstream tyrosines and precursors for downstream monolignols and phenylpropanoids, and it suggests that pathways for synthesizing phenol secondary metabolites from tyrosine are activated in Kintoki ginger.

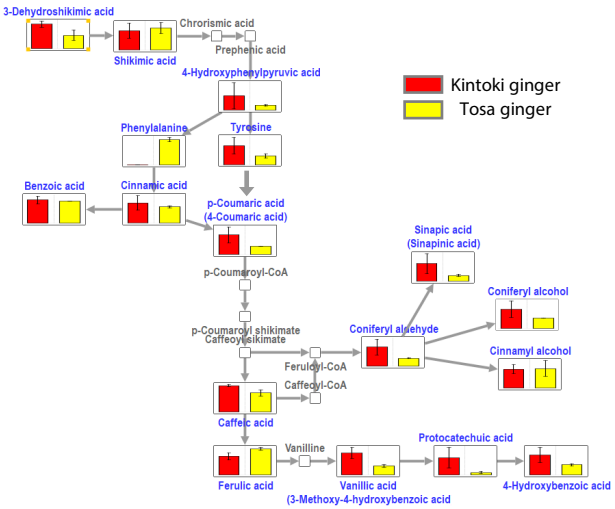

Fig. 6 Metabolic Mapping Analysis of Cinnamic Acid/Monolignol Pathway
Note that this metabolic map was created independently and is not included in the Multi-Omic Data Analysis Package.

■ Conclusion

Integrative analysis of the hydrophilic and hydrophobic metabolites of Kintoki and Tosa ginger found that there are clear differences between these ginger species. Kintoki ginger was found to contain many secondary metabolites, which have large amounts of medicinal and functionally beneficial components. This explains why Kintoki ginger has been used for medicinal purposes. In contrast, Tosa ginger contains more amino acids, which is presumably why it has been widely used for culinary purposes.

In addition, visualizing the activity of each metabolic pathway, based on a metabolic map of the primary metabolites, enabled the attribution of primary metabolites that were involved in the production of secondary metabolites.

The method described in this article offers the advantage of being able to analyze both hydrophilic and hydrophobic metabolic products using the same column. Furthermore, the Multi-Omic Data Analysis Package enabled consistent multifaceted statistical analysis of data, such as volcano plots, principal component analysis (PCA), and metabolic mapping.

■ References

1) Physiology of Plant Metabolism Beginning with the Fundamentals, Yodosha, 2018
2) Nishidono Y, Tanaka K. Effect of drying and processing on diterpenes and other chemical constituents of ginger. J Nat Med. 2023;77(1):118-127.

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