Compositional Comparison of Crops by On-line Solid-phase Derivatization SPE-GC/MS

Introduction

In conventional GC/MS analysis of metabolomics, extraction, lyophilization, and derivatization were complicated and time-consuming, and the data obtained tended to be highly inconsistent. We have dramatically shortened the time required, simplified the process, and achieved high accuracy through our proprietary “solid-phase derivatization” technology. Amino acids and organic acids are held in the solid phase by ion-exchange interactions, and then passed through acetonitrile to dehydrate and wash the solid phase. In this study, we attempted to compare the composition of vegetables and fruits using the SGI-M100, a fully automated system that performs these processes.

Sample

A. Spinach

Spinach contains high levels of oxalic acid, which binds with calcium in the body and can cause stones in the kidneys and urinary tract.

B. Tomato

It is used in ketchup, tomato sauce, pizza sauce, etc. because of its umami taste due to its very high concentration of glutamic acid and its acidity and moisture content.

C. Plum

It is characterized by its strong acidity and contains high levels of citric acid and other organic acids.

Pretreatment flow

Dry ice freezing and pulverizing

100~200 g of edible parts of crops were frozen and crushed with dry ice.

Sample 10 g
- Add Water 10 mL

Shaking
- Add ACN 20 mL

Shaking extraction 10 min

Centrifuge 3500 rpm, 5 min

Supernatant 500 µL
- Add ACN 500 µL

Shaking 37 ℃, 5 min

Cetrifuge 14000 rpm, 3 min
- Add 0.1N NaOH

Extract

On-line solid-phase derivatization

SPE-GC : SPL-M100

Vial

Automation

Pretreatment : 15 min.

- Conditioning ACN-Water(1/1) ACN
- **Flash-SPE ACXs**
- **Load[Retain] Supernatant** 50 µL Isolation
- Washing ACN-水(1/1)
- Dewater ACN
- Infiltration Methoxyamine/Pyridine
- SPD Methoximization, 3 min
- Infiltration MSTFA-Toluene(3/1)
- SPD TMS, 1 min
- Elution Hexane

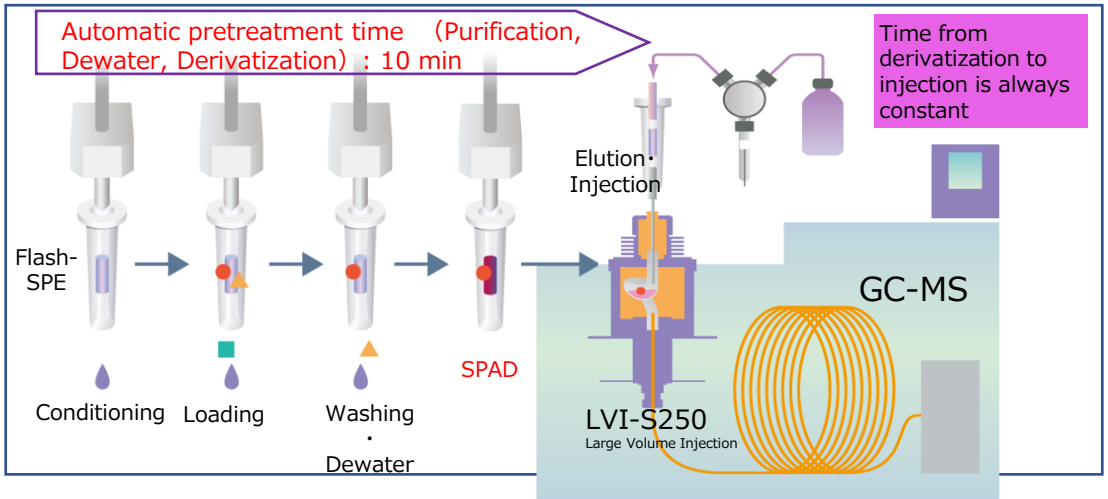
GC-MS Sprit 1:50

SPE-GC-MS system for metabolome analysis
SPL-M100 / GCMS-TQ8040NX

Analytical condition

PTV Injector	**LVI-S250 (AiSTI Science)**
Insert Type	Spiral Insert
Injector Temp.	220℃(0.5min)-50℃/min-290℃(16min)
GC-MS	
Inlet Mode	Split 1:50
Flow Mode	Constant Flow, 1 ml/min
Pre-Column	0.25mm i.d. x 1m
Column	Vf-5ms, 0.25mm i.d. x 30m, df;0.25µm
Oven Temp.	100℃(2min)-10℃/min-220℃-30℃/min-320℃
Trans. Temp.	290℃
MS Method	SCAN, m/z;70-470

Solid phase extraction to GC-MS injection process (fully automatic process)



SPL-M100
for SPE-GC system

Sample

vegetable
・fruit

Information

【Sample】
- Spinach
- Tomato
- Plum

【Target】
- Amino acids
- Amines
- Nucleobases,
- organic acids

AiSTI SCIENCE

Product

SPE-GC system
SPL-M100

Solid phase cartridge
Flash-SPE

GC Lage Volume Injection
LVI-S250

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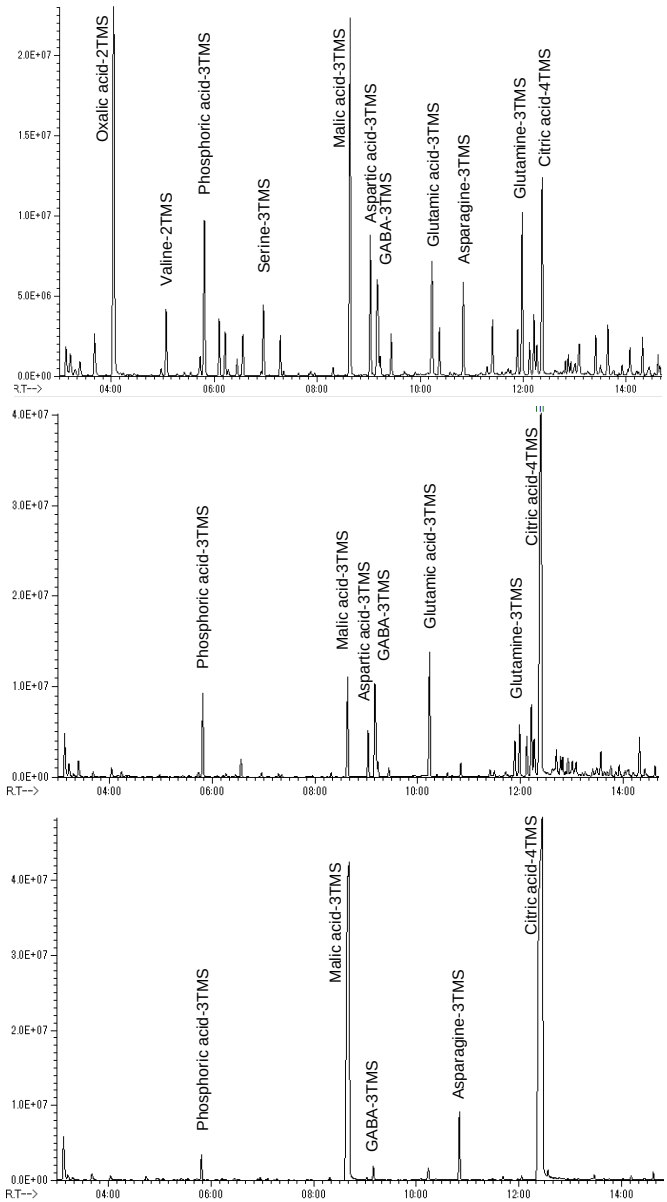
■SCAN total ion chromatograms for each crop using this method



■ Comparison of components of each crop

HOU:Spinach, TOM:tomate, UME:plum

【Results and Discussion】

Peak shapes and separations are also excellent for all three samples in the TIC. It can be inferred that washing, dehydration, and derivatization in the solid phase are efficiently performed. The solid-phase derivatization of this analytical method improves the accuracy and shortens the time required, and the automation of the method is also expected to increase efficiency. The method is expected to maximize the advantages of GC/MS, such as high separation, high qualitative capability, and a full database, by overcoming the complexity and long time required for pretreatment, which had been a weak point of the method.

In addition, a comparison of the component area of each sample show that the differences can be clearly determined. The data obtained from the highly accurate analytical method is expected to contribute to highly reliable analysis results, and can be utilized for future improvement of crop varieties and development of cultivation methods.