

# Metabolic profiling of soybean lipids by supercritical fluid chromatography/mass spectrometry

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## Introduction

Soybean is a well-known crop and it is widely used to produce many types of processed foods that are popular worldwide. In addition, it is important to understand the characteristics and adequate usage of soybean cultivars for applications in the food industry.

### Soybean processed food



Natto



Nimame



Tofu



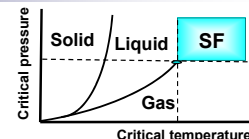
Edamame

Therefore, in this study, soybean lipid profiling was applied to discriminate soybean cultivars suitable for use in soybean processed foods, because a lipid profile is one of the specific factors used to discriminate and evaluate vegetable oils.

We applied supercritical fluid chromatography/mass spectrometry (SFC/MS) to soybean lipid profiling. Additionally, soybean cultivars were clustered according to their characteristics and usage by principal component analysis (PCA).

## Supercritical fluid (SF)

- Temperature and pressure above critical points
- Low viscosity
- High diffusivity
- Density is greatly transmutable continuously



SF has suitable properties for mobile phase of chromatography.

Supercritical carbon dioxide (SC-CO<sub>2</sub>)

Non-toxic, chemically inert, non-flammable, non-explosive, low response to the most detectors, non-polar

## Soybean cultivars

Breed	Contents	Suitability
Tamuraara	High protein and high lipid	Tofu and Nimame
Otsuru	Medium protein and medium lipid	Tofu and Nimame
Miyagishirome	High protein and low lipid	Nimame
Tambaguro-hyokei 3gou	Low protein	Nimame and Natto
Kimusume-ibaraki 1gou	Low protein and low lipid	Natto
Karikachi	High protein and high lipid	Natto
Natto-shouryu	High protein and low lipid	Natto
Murasaki-dadacha	Low protein	Edamame
Yukimusume	Low protein and high lipid	Edamame
Yukimusume2	Low protein and high lipid	Edamame
Horei	Low protein and low lipid	Unknown
Kinmame	Low protein and high lipid	Unknown

## Experiments

### Soybean lipid extraction

Twelve soybean cultivars were ground in a high-speed vibrating sample mill at 50 Hz for 1.5 min at room temperature.

Soybean powder (20 mg) was immersed in liquid N<sub>2</sub>, and the solution was ground for 1 min at 20 Hz in a ball mill mixer.

Soybean lipids were extracted with 500  $\mu$ l chloroform/methanol (2:1, v/v) and vortexed, sonicated, and centrifuged at 20000 g.

The supernatants were subjected to SFC/MS analysis.

### SFC/MS conditions

#### (a) SFC conditions (BERGER SFC™ Analytix, Waters)

Mobile phase : Carbon dioxide (CO<sub>2</sub>, 99.9% grade)  
 Modifier : Methanol with 0.1% (w/w) HCOONH<sub>4</sub>  
 Flow rate : 3 mL/min  
 Oven temperature : 35 °C  
 Back pressure : 10 MPa

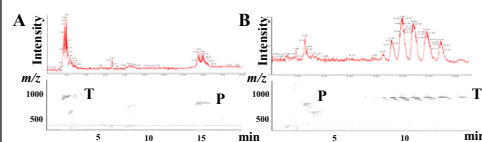
#### (b) MS conditions (ZQ2000, Waters)

Make up : Methanol with 0.1% HCOONH<sub>4</sub>  
 Scan range :  $m/z$  300 - 1200  
 Capillary voltage : 3.00 kV  
 Cone voltage : 30 V  
 Extractor voltage : 2.0 V  
 RF lenz voltage : 0.2 V  
 Source temperature : 120 °C

## Results and Discussion

### Soybean lipid profiling

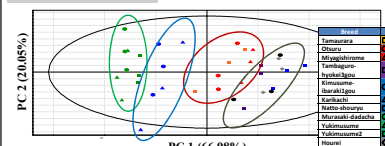
Diverse lipids extracted from soybean (*karikachi*) were analyzed successfully by SFC/MS.



(A) Cyano column (Berger cyano, Waters) T: triacylglycerol (TAG)  
 (B) ODS column (Nertsil ODS-3, GL Sciences) P: phosphatidylcholine (PC)

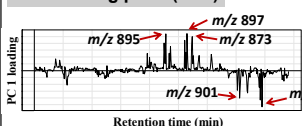
### PCA analysis

#### PCA score plots



Suitable soybeans used in the manufacture of different processed foods such as natto, tofu, edamame, and nimame were discriminated and grouped according to the cultivars by PCA.

#### PCA loading plots (PC1)



Triacylglycerol (TAG) was detected as the main variable for discrimination in the PCA loading plot.

### Soybean TAG profiling

For more effective discrimination of soybean cultivars, a high-throughput and high-resolution TAG profiling method was developed using SFC/MS with three Chromolith Performance RP-18e columns connected in series.

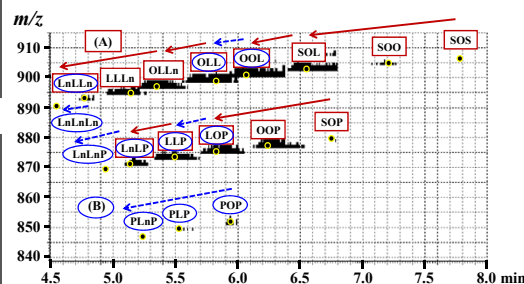
#### Chromolith Performance RP-18e 100 mm x 4.6 mm (Merck) x 3

- Silica type : High purity
- Structure : Monolithic shape
- Macropore size : 2  $\mu$ m
- Surface area : 300 m<sup>2</sup>/g
- Pore volume : 1 ml/g
- Surface modification : RP-18 endcapped
- Carbon content : 18 %
- Surface coverage : 3.6 mmol/m<sup>2</sup>
- pH range : 2 - 7.5
- Total porosity : > 81%

#### The programmed cone voltage fragmentation pattern of TAGs

TAG	$m/z$	Molecular ions ( $[M + NH_4]^+$ )	Fragmented ions ( $m/z$ )		
SOO	904.8	904.8	605.5 (SO)	603.4 (OO)	341.3 (S) 339.3 (O)
SOL	902.8	902.8	605.5 (SO)	603.5 (SL)	601.4 (OL) 339.3 (O) 337.3 (L)
OOL	900.8	900.9	603.5 (OO)	601.4 (OL)	339.3 (O) 337.3 (L)
OLL	898.8	898.8	601.4 (OL)	599.4 (LL)	339.3 (O) 337.3 (L)
OLLn	896.8	896.8	601.4 (OL)	599.4 (OLLn)	339.3 (O) 337.2 (Ln)
LLLn	894.8	894.8	599.4 (LL)	597.4 (LLn)	337.3 (L) 335.2 (Ln)
LnLn	892.7	892.7	597.4 (LLn)	595.4 (LnLn)	337.3 (L) 335.2 (Ln)
SOP	878.7	878.7	579.4 (SP)	577.4 (OP)	
OOP	876.8	876.8	577.4 (OP)		
LOP	874.8	874.8	577.4 (OP)	575.4 (LP)	
LLP	872.8	872.8	575.4 (LP)		
LnLP	870.7	870.7	575.4 (LP)	573.4 (LnP)	
LnLnP	868.7	868.7	573.4 (LnP)		

### TAGs on two dimensional map



A programmed cone voltage fragmentation method was used to identify the various TAGs. Cone voltages were increased in steps of 5 V from 30 to 90 V. As a result, the molecular structure of TAG was identified by the fragmented ions that had a specific value of  $m/z$ .

Small circle (o): peak top of each TAG.  
 Two pattern of TAGs arrangement.  
 Line arrows: a group of sn-1 fatty acid changed (box), ex) OLLn, LLLn, and LnLn (A).  
 Dotted arrows: a group of sn-2 fatty acid changed (circle), ex) POP, PLP, and PLnP (B).

The  $m/z$  values of the TAGs in the PCA loading plot were 902.8, 900.8, 896.8, 894.8 and 872.8, and they were respectively identified as SOL, OOL, OLLn, LLLn and LLP. This implies that soybean cultivars suitable for natto and edamame usually have SOL and OOL, and those suitable for nimame and tofu have OLLn, LLLn, and LLP.

## Summary

- ◆ A high-throughput and high-resolution lipid profiling method was established to discriminate soybean cultivars by SFC/MS.
- ◆ 12 soybean cultivars were separated by PCA according to their characteristics and usage. TAG was indicated as the main variable for discrimination.
- ◆ SFC/MS method for TAG profiling was established by using monolith ODS column. Each TAG was identified by 2D map and programmed cone voltage fragmentation.