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## Abstract

Soy sauce is a seasoning that is being increasingly used worldwide from Asia due to increased interest in health and unique savory taste. In our research, by applying non-targeted metabolite profiling approach, we interrogated chemical compositional difference between traditionally fermented soy sauce and commercial one that is made from mixture of soy and wheat. The result showed a range of amino acids, hydroxyl acids and saccharide are remarkably abundant in the traditional soy sauce compared to commercial soy sauce. So, we suppose that flavor and nutrients difference between traditionally fermented soy sauce and commercial one. Further, we explored ripening-period dependent dynamics in metabolite contents, which may play key role in alleviating uncomfortable flavor thus improving 'good taste'. We monitored temporal changes in metabolites levels along with 1 ~ 5 years of Korean traditional soy sauce. The result demonstrated a total of 58 were significantly down-regulated and dihydroxyacetone is the only metabolite showing higher abundance in longer ripening time. Dihydroxyacetone is an intermediate of Maillard reaction and known to be hundreds of different flavor compounds are created, so flavors and cents are rich and depth, and alleviates bitter. These findings will be utilized for the predictive integrated analysis and understanding of the total metabolites change of Korean traditional soy sauce, coupled to liquid-chromatography mass-spectrometry analysis and sensory test. Based on these results, in the future we expect that metabolomics will be used in a variety of ways with food science, such as improving food quality.

## Materials

### Soy sauce

Commercial Soy sauce	Traditional Korean Soy sauce					
	6Month	2 Month	6 Month	1 Year	3 Year	5 Year

### Metabolomics

metabolomics is the "systematic study of the unique chemical fingerprints that specific cellular processes leave behind, it has the advantage of being able to analyze and interpret the type and amount of metabolism that changes in various conditions.



5 Year Soy sauce oak

### Objective

1. Profiling primary metabolites depending on fermentation period and Screening specific metabolites comparing Korean traditional soy sauce with commercial soy sauce
2. Investigating part-specific chemical composition in Korean traditional soy sauce
3. Exploring secondary/functional compounds using LC-MS/MS with subsequent function test (e.g. radical scavenging capacity)

## Methods

### Mass-spectrometry analysis

**Sample Extraction (matyash method)**

30 µl Sample + 228 µl Cold Methanol  
 Adding 750 µl MTBE  
 Vortexing for 10sec & shaking for 10min with 200rpm  
 Adding 187 µl D.W  
 Shaking the solution and separate for 10 min

**LC Sample**  
 300 µl sample Upper phase + 300 µl 0.1% Formic acid in ACN  
 Filter with 0.2 µm Nylon6 springs filter  
 Analyze using HPLC

**GC Sample**  
 200 µl Sample Lower Phase  
 Speed vac

**Derivatization**  
 Adding 5µl of a solution of 40mg/mL of 88% pure methoxyamine hydrochloride in pyridine at 30°C for 60min  
 Adding 45µl of MSTFA (N-methyl-N-(trimethylsilyl)-trifluoroacetamide)  
 Adding 2µl of FAME (Fatty acid methyl ester)  
 Analyze using GC

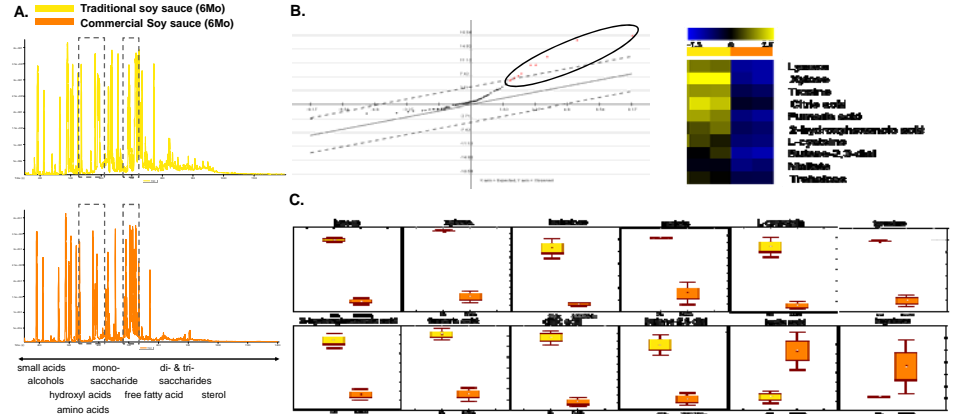
### Metabolite profiling

Metabolites were analyzed with Leco GC-TOF mas spectra acquisition from m/z 85 to 500 at 17 spectra/s and 1700V detector voltage. The injector was operated in splitless model; the split vent was opened after 25s. Samples were injected into the 50°C injector port, which was ramped to 250°C in 12°C/min and held for 3min.

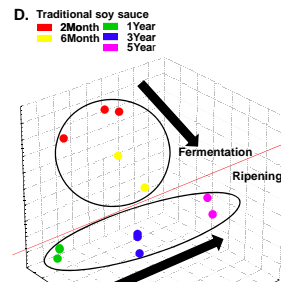
## Conclusions

1. We find that 17 significantly up-regulated and one down-regulated compounds in traditional soy sauce compared to commercial one.
2. Among the altered up-regulation compounds, amino acids, saccharide and hydroxyl acids are remarkably abundant in the traditional soy sauce.
3. In according to increased of ripening time, we discovered that dihydroxyacetone is the only up-regulated metabolite while most compounds are down-regulated.
4. Also, we recognized that dihydroxyacetone was up-regulated and sugar and amino group were down-regulated by non-targeted analysis. It is implicated that the Maillard reaction occurs in Soy sauce and means flavors and cents are more rich and depth whereas bitter is alleviate.

## Results

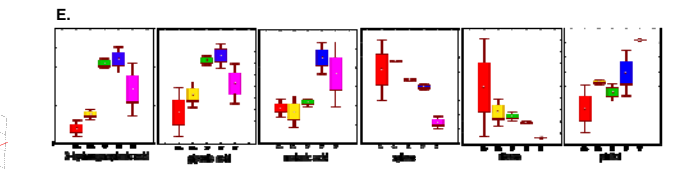


**A.** The MS chromatogram (Total Ion Chromatogram) of soy sauce shows the very distinctive pattern from Traditional and Commercial. Particularly hydroxyl acid, amino acid, and saccharides were found the most significantly different between Traditional and Commercial.



**B.** Significance analysis of microarray (SAM) were performed for screening genotype-specific metabolite alteration. False discovery rate was maintained as zero. Metabolites located up-right (red circle) indicates significantly up-regulated ones with existence of gene X (Expected score < Observed score). The detailed list is displayed in the table.

**C.** Relative abundances of statistically different compounds by SAM. Some of exemplary metabolites of SAM were depicted by box-whisker plot to present relative abundance and dynamic changes.



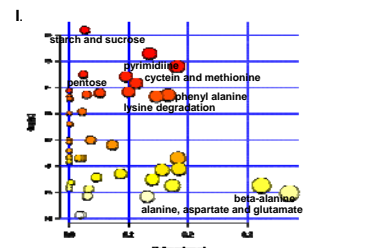
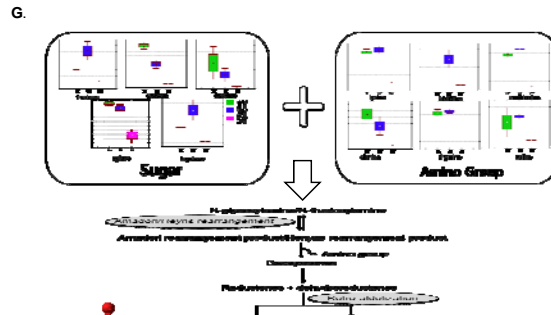
**F.**

	2Mo	6Mo	1Y	3Y	5Y	2Mo	6Mo	1Y	3Y	5Y	2Mo	6Mo	1Y	3Y	5Y
2Mo	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
6Mo	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
1Y	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
3Y	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
5Y	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000

**D.** Supervised multivariate statistics using partial least square (PLS) analysis of GC-TOF MS data. Differential metabolic phenotypes were captured by SA treatment in dose-responsive manner. In addition, the score plot demonstrated time-dependent separation. When the dataset was divided into Fermentation and Ripening stages

**E.** Relative abundances of statistically different compounds by one way ANOVA. Some of exemplary metabolites of ANOVA were depicted by box-whisker plot to present relative abundance and dynamic changes.

**F.** ANOVA and post hoc tests adjusted by multiple comparisons using Scheffe test



Pathway Name	P	Impact
Starch and sucrose	1.03E-04	0.02647
Pyrimidine	2.53E-04	0.13498
Cysteine and methionine	4.16E-04	0.1814
Pentose	5.60E-04	0.02401
Lysine degradation	0.0012922	0.14675
Phenylalanine metabolism	0.0012919	0.18052
beta-Alanine	0.003999	0.32319
Alanine, aspartate and glutamate	0.051875	0.37037

## References

1. Lee do Y, Kind T, Yoon YR, Fiehn O, Liu KH (2014) . Comparative evaluation of extraction methods for simultaneous mass-spectrometry analysis of complex lipids and primary metabolites from human blood plasma
2. Vitali Matyash, Gerhard Liebisch T, Teymuraz V, Kurzhachia, Andrej Shevchenko , and Dominik Schwudke (2008) , Lipid extraction by methyl-tert-butyl ether for high-throughput lipidomics