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Application of metabolomics for discrimination and sensory predictive modeling of food products

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Omics

(*Science by means of*
"Total profile" of biomaterials)

Object	Total profile	Omics
Gene	Genome	Genomics
Transcript(mRNA)	Transcriptome	Transcriptomics
Protein	Proteome	Proteomics
Metabolite	**Metabolome**	**Metabolomics**

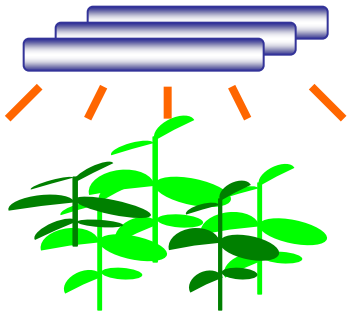
What is metabolomics?

Metabolomics is a research field concerned with the comprehensive analysis of metabolites and other small molecules in biological samples

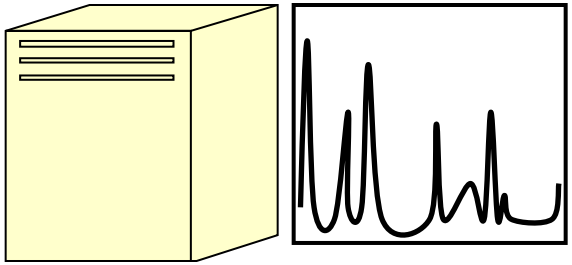
Metabolomics involves the analysis of a wide variety of biological samples (animal/plant/microbe cells, food, pharmaceuticals etc) using various instruments and techniques (chromatography, mass spectrometry, data analysis etc).

The applications of metabolomics are as diverse as understanding biological function, assessment of drug toxicity, evaluation of food processing methods and others, many of which are directly linked to the improvement of our everyday lives.

“Metabolomics”is an interdisciplinary research between Bioscience, Analytical chemistry and Informatics



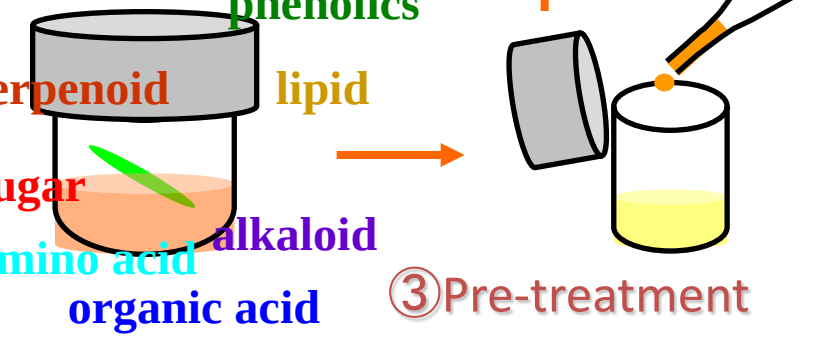
①Biomaterials



④Analysis

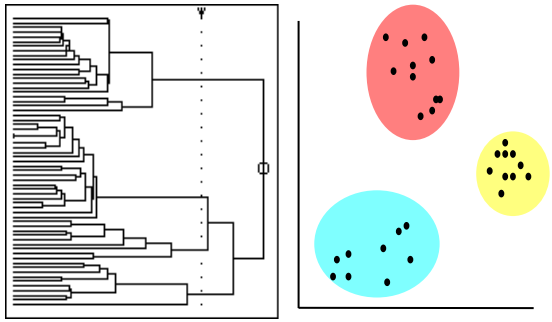
	代謝産物x_1	···	代謝産物x_n
No.1	x_{11}	···	x_{1n}
.	.		.
.	.		.
.	.		.
No.m	x_{m1}	···	x_{mn}

⑤Matrics formation



②Sampling

③Pre-treatment



⑥Data Mining

⑦Feadback

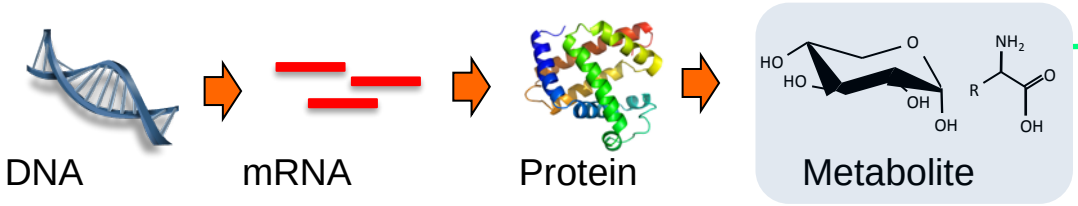
Metabolomics in food science

FOOD

Chemical pools consisting of thousands of metabolites and its interaction in a form of solid, semi-solid and liquid matrix

Metabolomics

Exhaustive profiling of metabolites



Final readouts of metabolism

Physiological Phenomenon

Food quality evaluation

- Determination of the ratio of chemical constituents
- Seeking specific marker
- Global (physical & chemical) analysis

Wishart, *Trends Food Sci Technol*, 2008, Cordella et al, *J Agric Food Chem*, 2002

The power of metabolomics

Biological samples

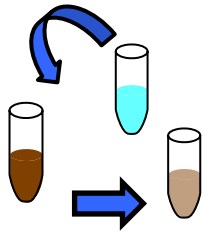
~ ¥ 3000/100gr

Kopi Luwak

~ ¥ 300/100gr

Regular coffee

Extraction & Derivatization



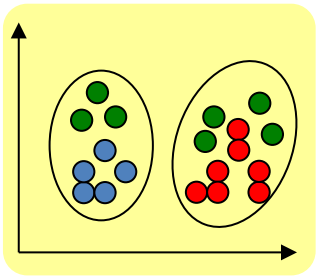
- Methanol, water, chloroform = 5/2/2
- Methoxyamine hydrochloride & MSTFA

Metabolite measurement (GC/MS, GC/FID)

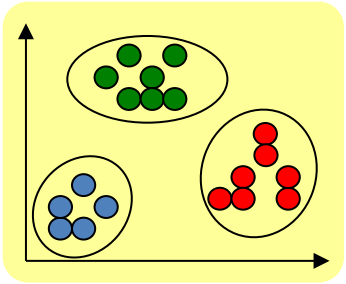
Peak table list (sample *vs* metabolite) Data preprocessing

Multivariate data analysis

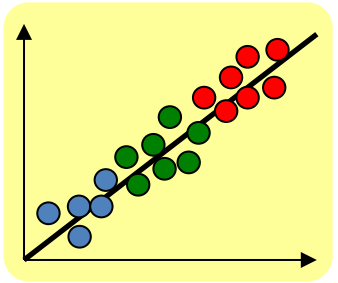
Discriminative



Informative



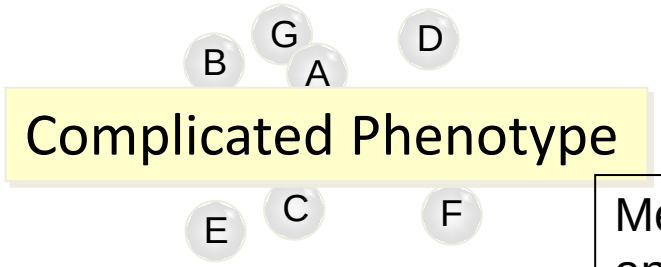
Predictive



Seeking specific marker (and its expandability)

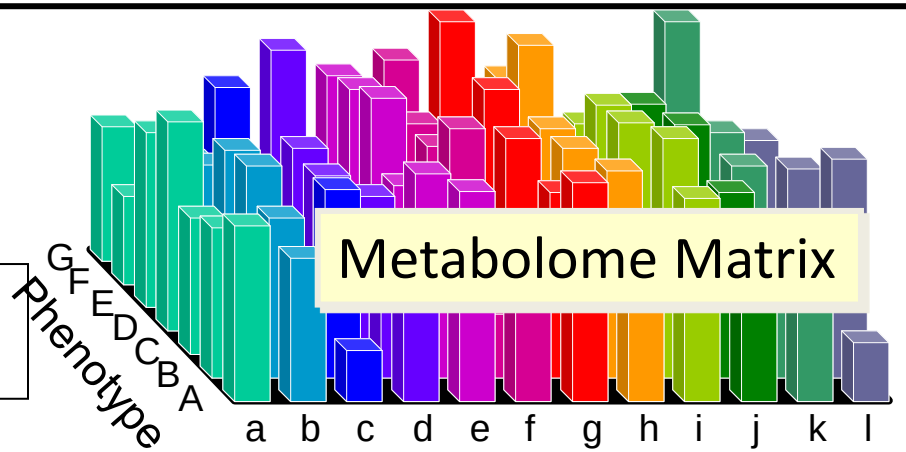
Determination of the ratio of chemical constituents

Concept of predictive metabolomics

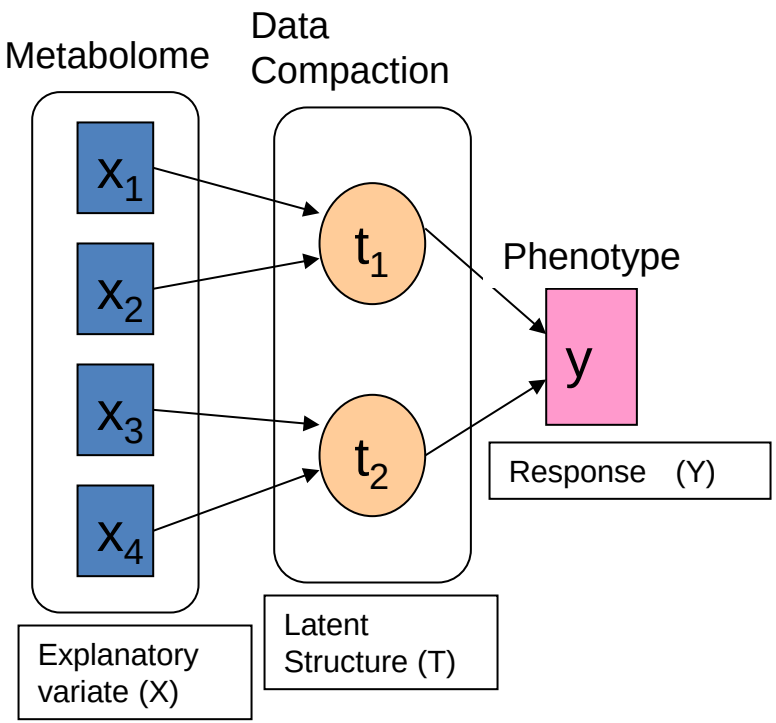


Complicated Phenotype

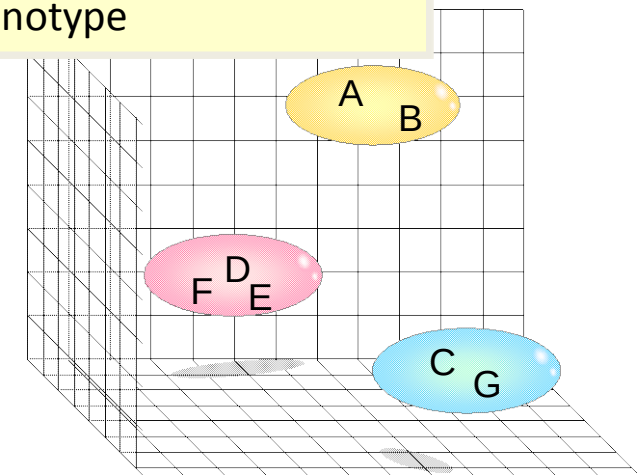
Metabolome analysis



Data mining by multi-variate analysis



Cluster separation depending to phenotype



Metabolome based Prediction of Phenotype

Metabolic profiling of food

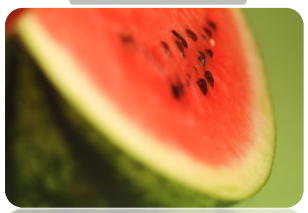
http://www2.fukusaki-lab.com/

Research of food

Basically, there are 4 steps for research using technique of metabolic profiling

1. **Sample preparation**
2. **Acquisition of food quality data such as sensory evaluation**
3. **Acquisition of components data**
4. **Data analysis by using multivariate analysis**

Food



Sensory analysis (Quality evaluation)

Analysis by instruments

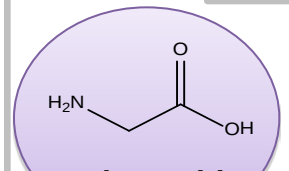
Functions

- **Taste**
- **Flavors**
- **Palatability**
- **Grades**

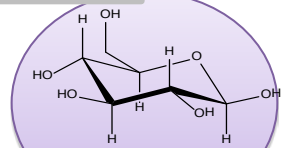
etc.

Analysis of relation between functions and components

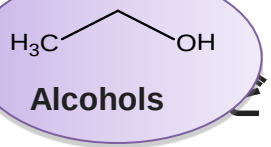
Components



Amino acids



Sugars

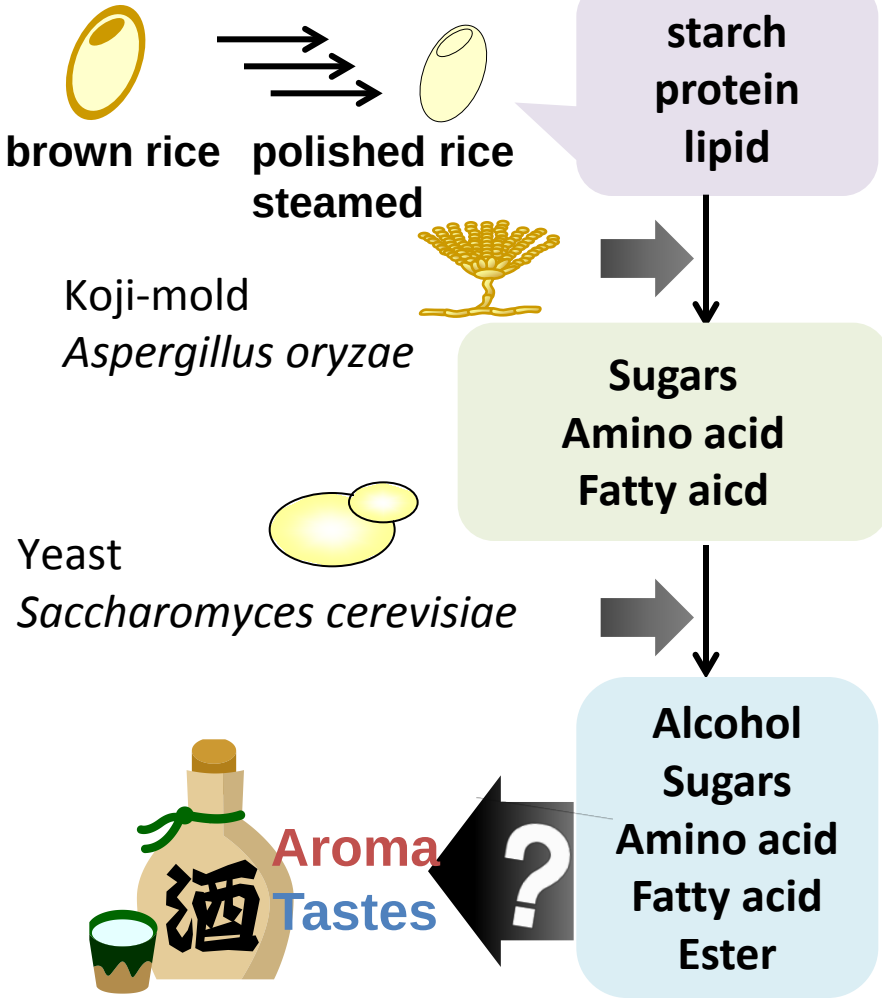


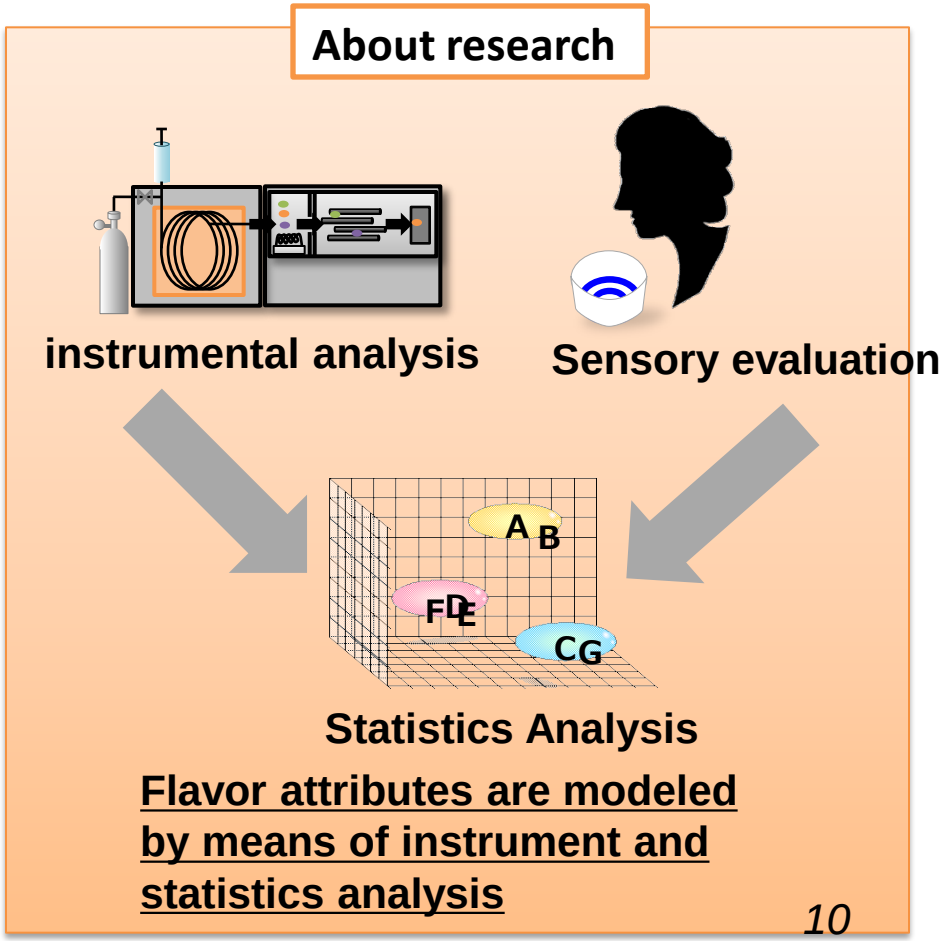
Alcohols

etc.

Components profiling of *Sake* (Japanese rice-wine)

Sake, a Japanese rice wine brewed with *koji* and yeast, is a traditional alcoholic beverage. Various components are generated in fermentation process and contribute to flavor. The history of the research about sake is long but it has uncleared abut the relationship between components and sake flavor such as "*Kire*" or " smoothness"





Prediction of green tea ranking by instruments

A set of ranked green tea samples from a Japanese commercial tea contest was analyzed with the aim of creating a reliable quality-prediction model. In addition, metabolites that play an important role in green tea's grade classification were identified.

Green tea

Sensory analysis

Ranking	sample
1	Green tea A
2	Green tea B
3	Green tea C
⋮	⋮
64	Green tea X

Analysis by instruments


FT-IR

or


NMR

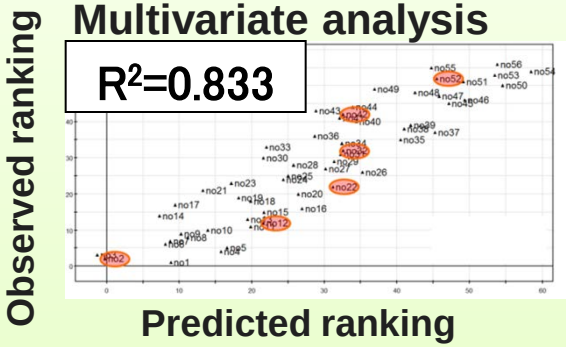
or


LC/MS

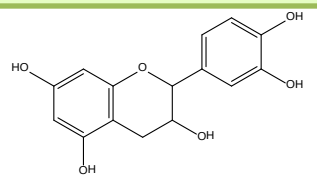
or


GC/MS

Multivariate analysis



It is possible to predict green tea ranking by instruments



Epicatechin

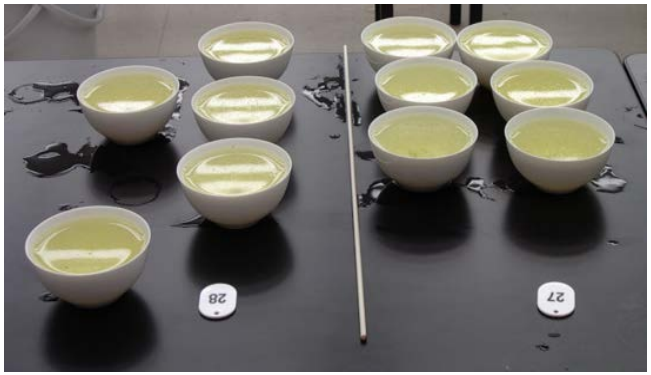
High rank green tea contains epicatechins.

Pongsuwan, W., *et. al.*, J. Agric. Food Chem., 55, 2, 231-236(2007)
Tarachiwin, L., *et. al.*, J. Agric. Food Chem., 55, 23, 9330-9336 (2007)
Ikeda, T., *et. al.*, J. Agric. Food Chem., 55, 24, 9908-9912 (2007)
Pongsuwan, W., *et. al.*, J. Agric. Food Chem., 56, 22, 10705-10708 (2008)

Sensory test of Green Tea by skilled panelist

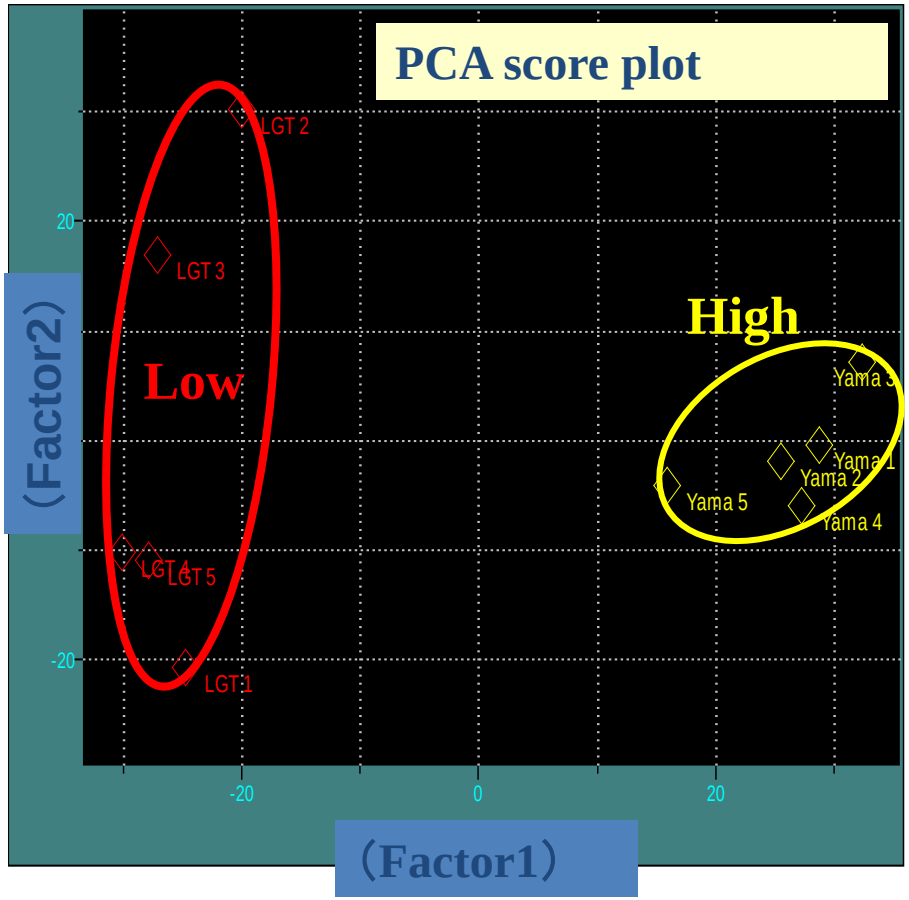
Taste

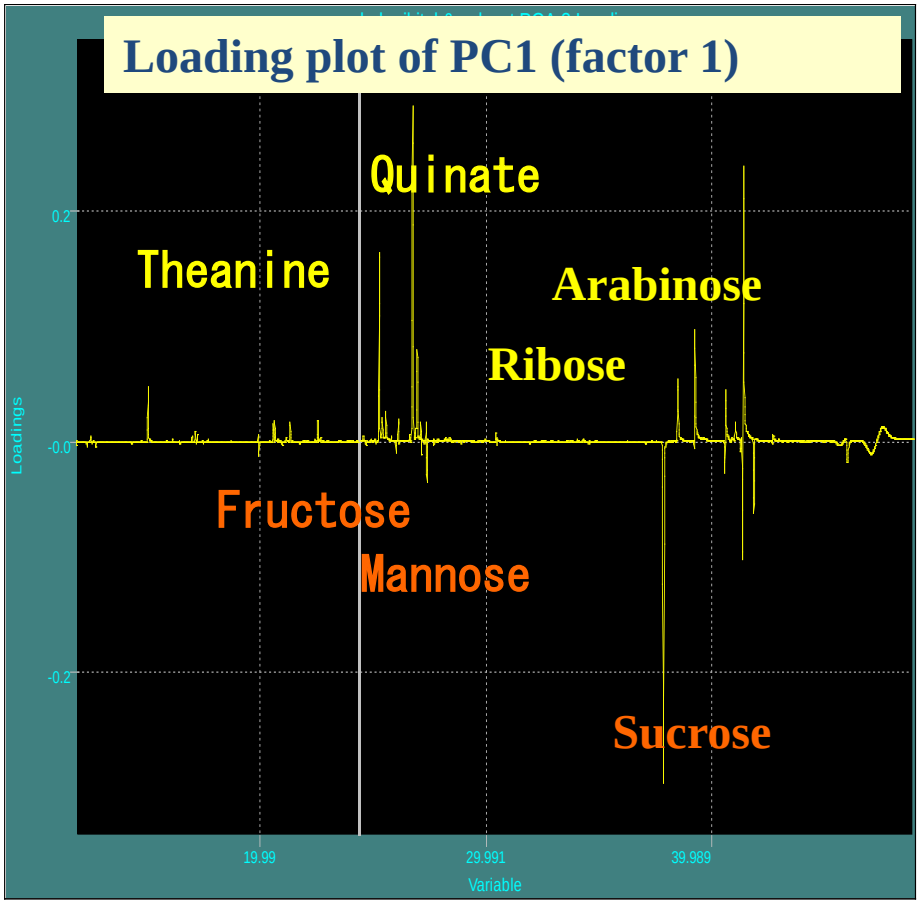
Aroma



Color

Comparison of high grade green tea and low grade one





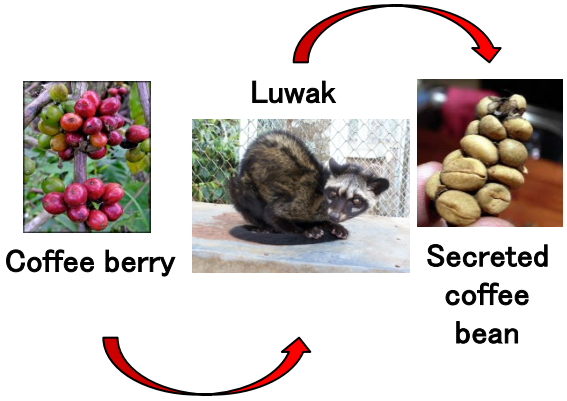
High grade tea and Low grade tea are clearly separated by GC/MS based PCA analysis.

Yellow: high amount in high grade tea

Orange: high amount in low grade tea

GC/MS-based metabolic profiling of civet (Luwak) coffee

- Luwak coffee made from coffee berries that have been eaten by Asian palm civet or Luwak.
- Luwak coffee acclaimed as *the world's most expensive coffee,* owing to unique flavor and unexpected processing.
- However, practical method for authentication is not available.



Objective

To develop rapid and robust method for the authenticity assessment of civet (Luwak) coffee

Strategy

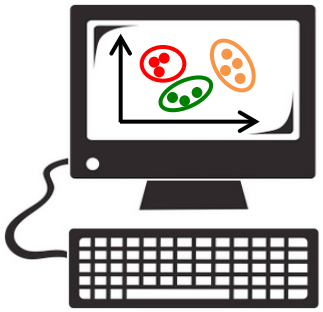

Ground coffee bean


Roast bean

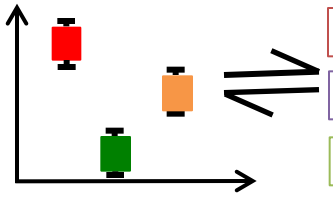
Raw bean (washed & dried)


Metabolic profiling (GC-Q/MS)

Identification of discriminant marker


Multivariate data analysis (PCA, OPLS/DA)

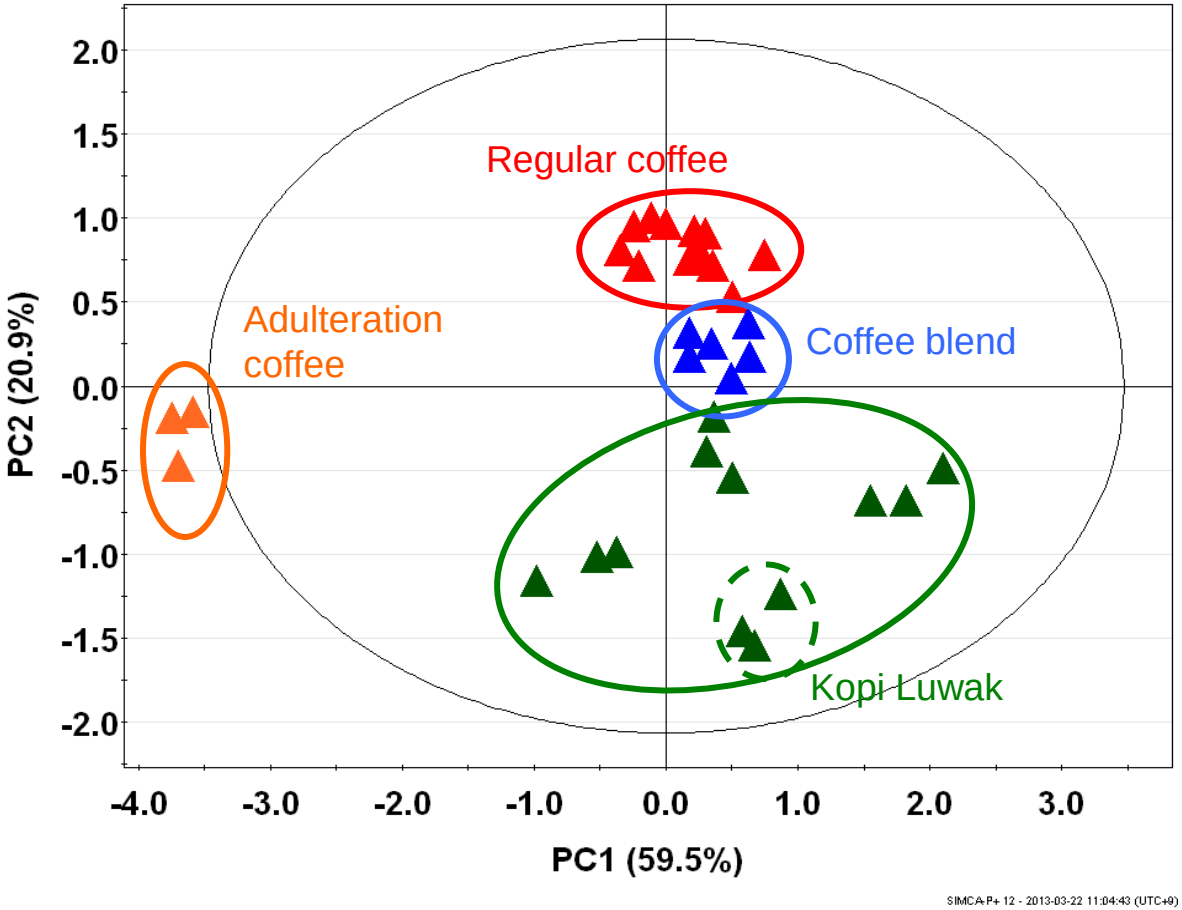
Validation of applicability of discriminant marker to distinguish various commercial coffee products



U Jumhawan, *et. al.*, *J. Agric. Food Chem.*, **61**, 33, 7994-8001 (2013)

Results and Discussion

Validation of the applicability of discriminant marker



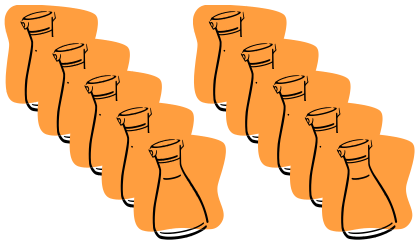
Sample replication (n) = 3
Scaling = Pareto
Authentic Kopi Luwak

Principal component analysis for validation

Samples were well-clustered in PCA generated by projecting discriminant marker candidates as inclusion list

Analysis of the relation between taste and components of soy sauce

Soy sauces



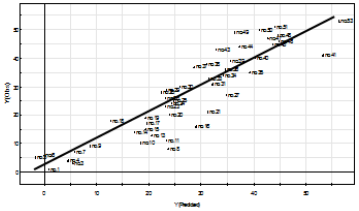
Sensory analysis

Umami
Sweetness
Saltiness
etc.

Intensity of each attribute

Multivariate analysis

Projection to Latent Structure (PLS)

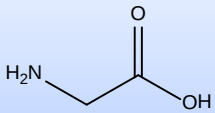


- Prediction of each intensity from metabolome data
- Identification of highly correlated compounds

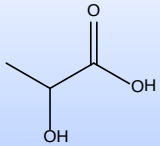
Metabolome data

GC/MS&LC/MS

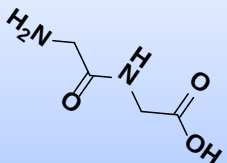
Low-molecular hydrophilic compounds



Amino acids



Organic acids



dipeptide etc.

Yamamoto, *et. al.*, *J. Biosci. Bioeng.*, **114**, 170-175 (2012)
Yamamoto, et al., *J. Biosci. Bioeng (in press)*

Metabolomics contribution to banana research

Metabolites changes in banana ripening process under microgravity condition

Sampling

CBEF(Micro-G)

CBEF(1G)

Banana in different stage

Sample return to earth

Waring Blender WSB 33E

GCMS-QP2010 Ultra (Shimadzu)

Monosaccharide, sugar alcohol, organic acid, fatty acid, amino acid

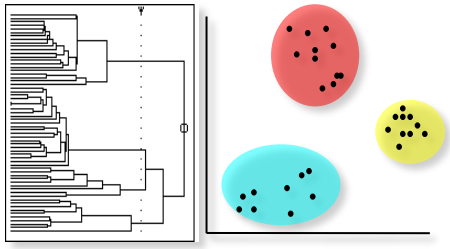
UHPLC-UV (Shimadzu)

Sugar, nucleic acid, protein, fatty acid

- Key metabolic steps in banana ripening process
- Dynamicity of metabolite changes of banana ripening process in space environment

feedback

results



Publications on food research

Green tea

- **Ono D, Bamba T, Oku Y, Yonetani T, Fukusaki E., “Application of Fourier transform near-infrared spectroscopy to optimization of green tea steaming process conditions. J Biosci Bioeng. 2011 Sep;112(3):247-51. Epub 2011 Jun 2.**
- **Jumtee, K., Bamba, T. and Fukusaki, E. "Fast GC-FID based metabolic fingerprinting of Japanese green tea leaf for its quality ranking prediction." *J Sep Sci* 32(13): 2296-2304.(2009) Epub ahead of print on 2009/06/30**
- **Pongsuwan, W., Bamba, T., Harada, K., Yonetani, T., Kobayashi, A. and Fukusaki, E. "High-Throughput Technique for Comprehensive Analysis of Japanese Green Tea Quality Assessment Using Ultra-performance Liquid Chromatography with Time-of-Flight Mass Spectrometry (UPLC/TOF MS)." *J Agric Food Chem* 56(22): 10705-10708.(2008)**
- **Ikeda, T., Altaf-Ul-Amin, M., Prvin, A. K., Kanaya, S., Yonetani, T. and Fukusaki, E. "Predicting Rank of Japanese Green Teas by Derivative Profiles of Spectra Obtained from Fourier Transform Near-Infrared Reflectance Spectroscopy." *Journal of Computer Aided Chemistry* 9: 37-46.(2008)**
- **Pongsuwan, W., Bamba, T., Yonetani, T., Kobayashi, A. and Fukusaki, E. "Quality Prediction of Japanese Green Tea Using Pyrolyzer Coupled GC/MS Based Metabolic Fingerprinting." *J Agric Food Chem*. 56(3): 744-750 (2008)**
- **Ikeda, T., Kanaya, S., Yonetani, T., Kobayashi, A. and Fukusaki, E. "Prediction of Japanese green tea ranking by fourier transform near-infrared reflectance spectroscopy." *J Agric Food Chem* 55(24): 9908-9912.(2007)**
- **Tarachiwin, L., Ute, K., Kobayashi, A. and Fukusaki, E. "(1)H NMR based metabolic profiling in the evaluation of Japanese green tea quality." *J Agric Food Chem* 55(23): 9330-9336.(2007)**
- **Pongsuwan, W., Fukusaki, E., Bamba, T., Yonetani, T., Yamahara, T. and Kobayashi, A. "Prediction of Japanese green tea ranking by gas chromatography/mass spectrometry-based hydrophilic metabolite fingerprinting." *J Agric Food Chem* 55(2): 231-236.(2007)**

Soy sauce, water melon and Luwak coffee

- **Yamamoto S, Bamba T, Sano A, Kodama Y, Imamura M, Obata A, Fukusaki E. "Metabolite profiling of soy sauce using gas chromatography with time-of-flight mass spectrometry and analysis of correlation with quantitative descriptive analysis." *J Biosci Bioeng.* 2012 Aug;114(2):170-5.**
- **Yamamoto S, Bamba T, Sano A, Kodama Y, Imamura M, Obata A, Fukusaki E. "Metabolite profiling of soy sauce using gas chromatography with time-of-flight mass spectrometry and analysis of correlation with quantitative descriptive analysis." *J Biosci Bioeng. in press***

- **Tarachiwin, L., Masako, O. and Fukusaki, E. "Quality evaluation and prediction of *Citrullus lanatus* by 1H NMR-based metabolomics and multivariate analysis." *J Agric Food Chem.* 2008 56(14): 5827-5835.**

- **umhawan U, Putri SP, Yusianto Y, Marwanni E, Bamba T, Fukusaki E. "Selection of Discriminant Marker for Authentication of Asian Palm Civet Coffee (Kopi Luwak): A Metabolomics Approach." *J Agric Food Chem.* 2013 ;61(33):7994-8001**

Cheese

- **Ochi, H., Naito, H., Iwatsuki, K., Bamba, T. and Fukusaki, E.: Metabolomics-based component profiling of hard and semi-hard natural cheeses with gas chromatography/time-of-flight mass spectrometry, and its application to sensory predictive modeling. *J. Biosci. Bioeng*., 113, 751−758（2012）**

- **Ochi, H., Bamba, T., Naito, H., Iwatsuki, K. and Fukusaki, E.: Metabolic fingerprinting of hard and semi-hard natural cheeses using GC/FID for practical sensory prediction modeling. *J. Biosci. Bioeng.*, 114, 506 − 511（2012)**

- **Ochi, H., Bamba, T., Naito, H., Iwatsuki, K. and Fukusaki, E.: Monitoring the ripening process of Cheddar cheese based on hydrophilic component profiling using gas chromatography-mass spectrometry. *J. Dairy. Sci.*, 96, 12, 7427-7441（2013)**

Final Remarks

Metabolomics is a very useful tool for various fields of science and engineering

It is up to you where and how to use this new technology. Use your imagination and you will get new and exciting discovery using metabolomics

Thank you for your kind attention