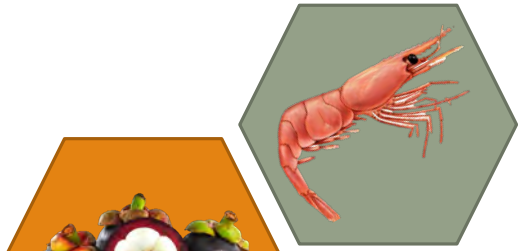
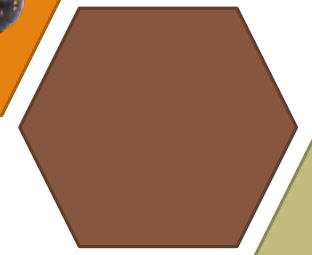
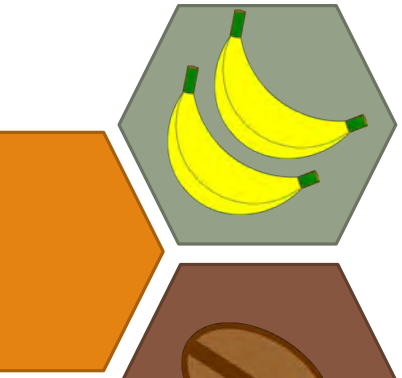
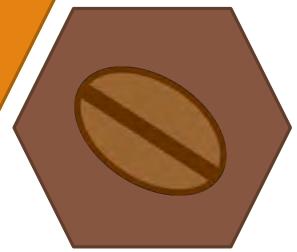
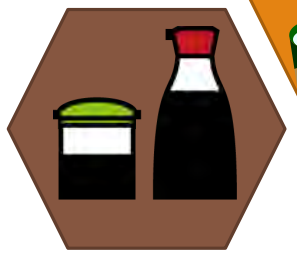




Food Metabolomics







Food

Change in food component

Food processing

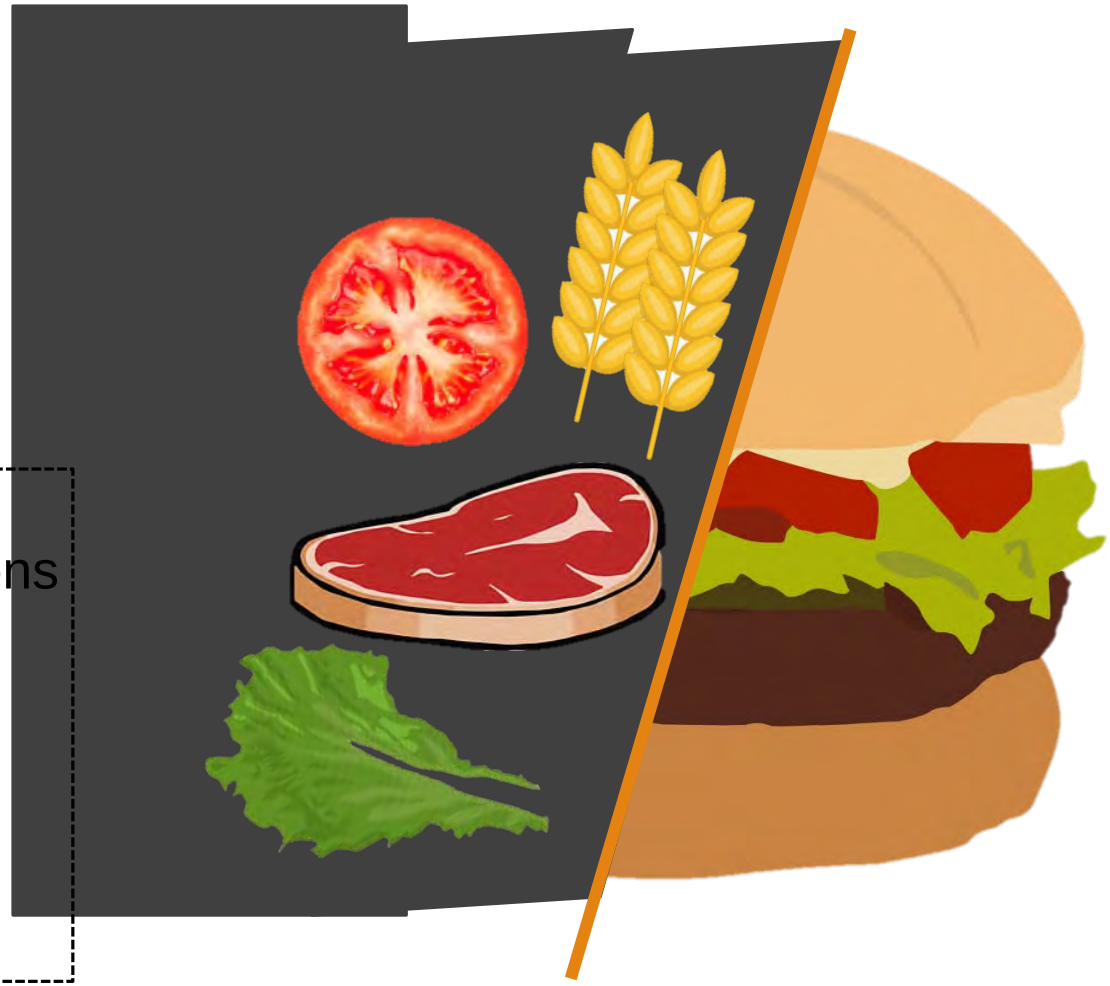
Factors

Environmental conditions

Cooking process

Handling process

Storage



Contributed to various

Sensory attributes

Taste

Aroma

Appearance

Texture

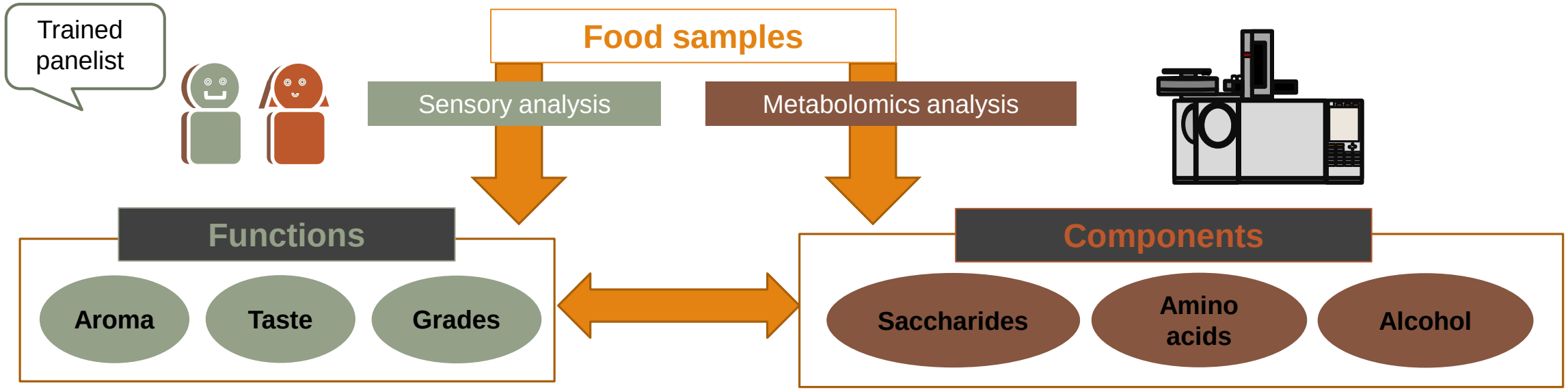
Food Metabolomics

Metabolomics as a comprehensive study to analyze small molecules in samples can give discriminative, predictive, and informative information that can be applied to obtain better control and understanding of production.

Metabolomics analysis

Predictive analysis

To predict various phenotype through statistical model (Multivariate Data Analysis/MVDA) by utilizing metabolome data as an explanatory variable.



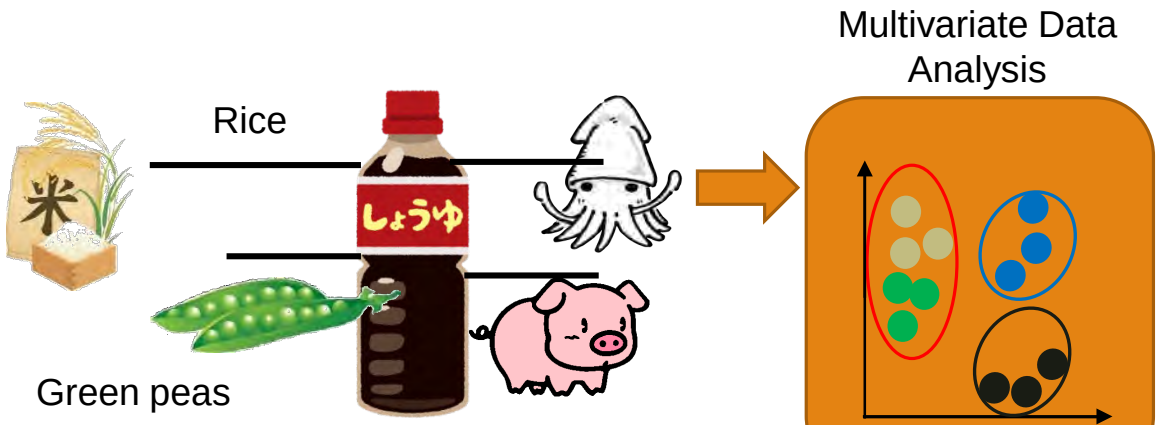
Analysis of relation between functions and component

Food Metabolomics

Metabolomics analysis

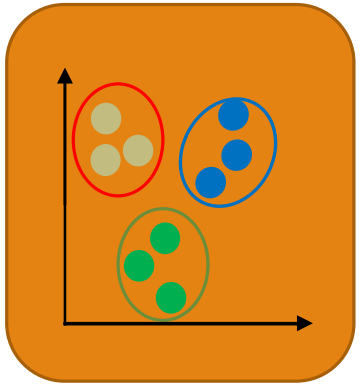
Discriminative analysis

To investigate the differences between sample populations (fingerprinting) without necessarily obtaining the intrinsic information of sample



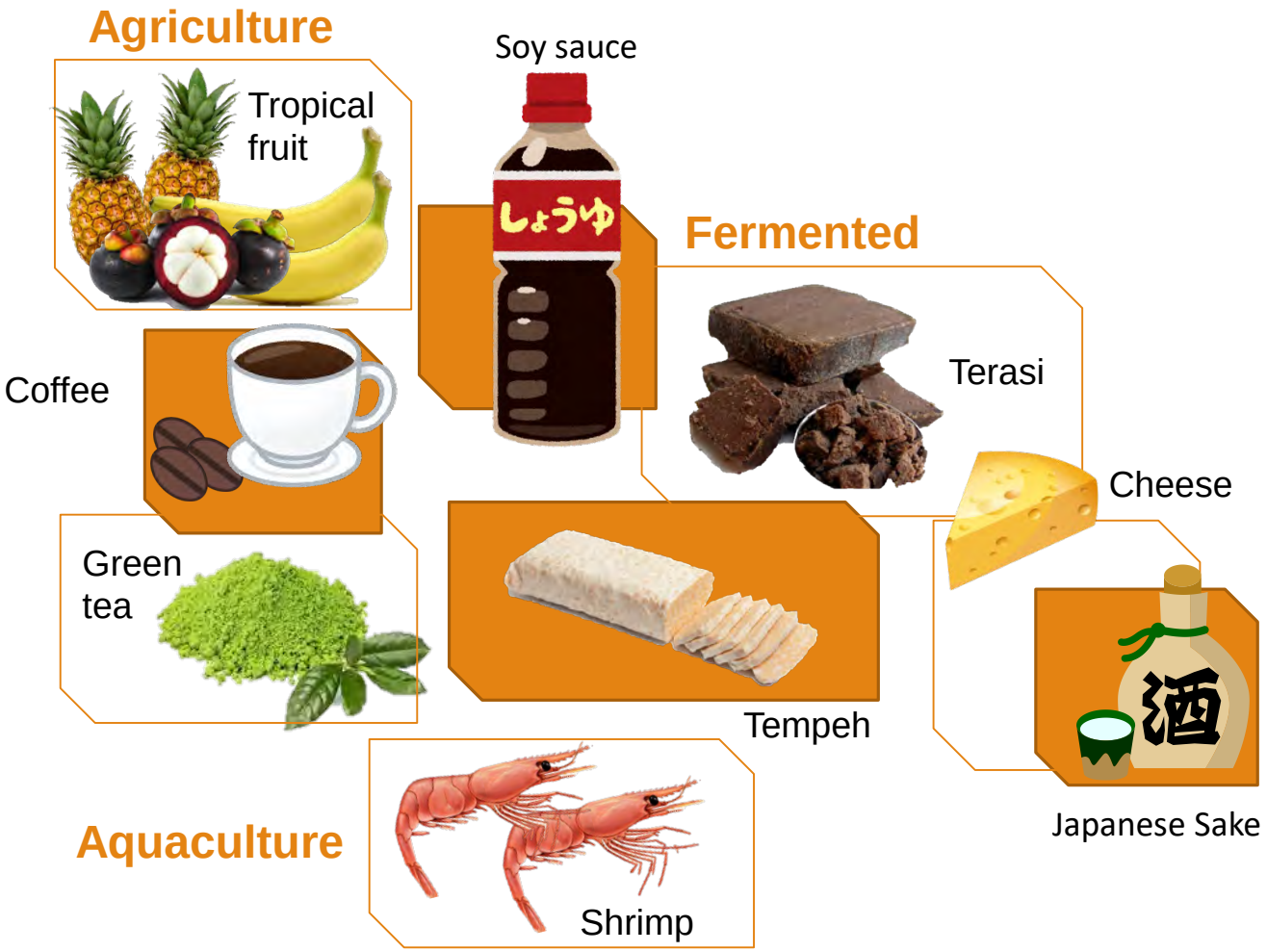
Informative analysis

To characterize and identify target compound. Possible pathways, discovery of novel bioactive compounds, discovery of biomarkers, creation of specialized metabolite databases, and metabolites functionality studies can also be carried out by informative metabolomics



Metabolomics approach of food products

Metabolomics can be applied to various food product such as natural food product (agriculture, aquaculture) or processed/fermented food product.



Food metabolomics application

Component analysis

Quality improvement

Authenticity assessment

Diet monitoring

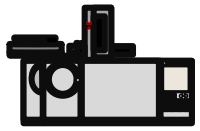
Metabolomics for fruit ripening studies

- Banana and mangosteen as a climacteric fruits depend on ethylene bursts that trigger metabolite production or breakdown to influence fruit color, taste, and firmness.
- Color changes are frequently used as a common ripening parameter

Samples collection

Metabolomics analysis

Explanatory variable



Time course metabolome analysis (GC/MS)

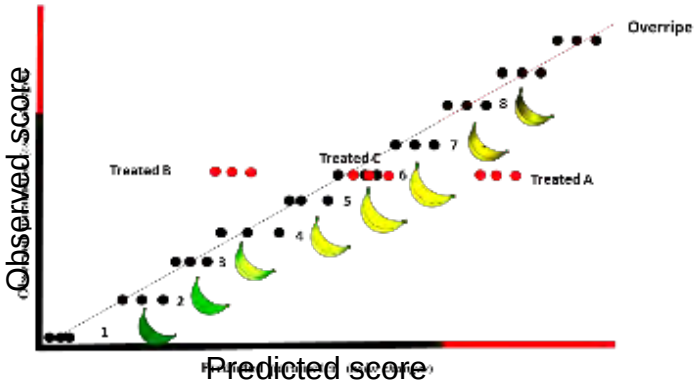
Color measurement

Response variable

Colorimeter (CM-2500)

Data Analysis

- Correlate the color changes and metabolite profiling data by constructing PLS regression model
- Predict the contributed metabolites of fruit ripening process



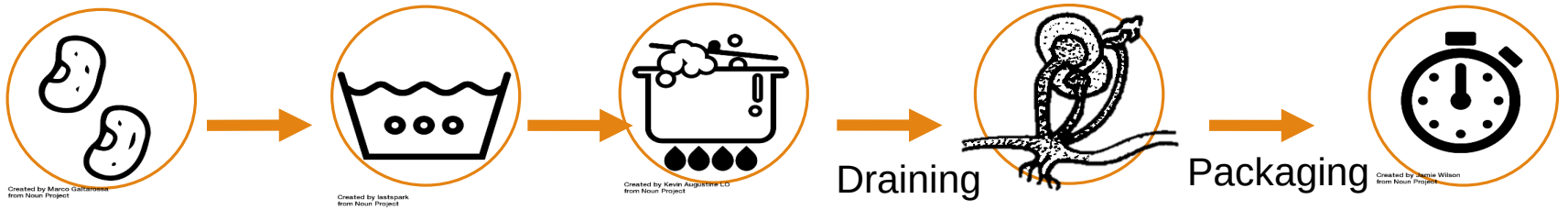
Provide important clues for understanding ripening mechanisms and identify bio-markers to improve post-harvest technology of fruits

Parijadi AAR, et al. J Biosci Bioeng. 2017 Sep 29. pii: S1389-1723(17)30475-9. doi: 10.1016/j.jbiosc.2017.08.013. [Epub ahead of print]

Metabolomics for tempeh studies

- Tempeh is Indonesian fermented food that is made by inoculating fungal starter to cooked soybean
- Tempeh is known as a meat alternative and main protein source in Indonesia

Tempeh production method



Soybean Dehulling*

Soaking

Boiling

Inoculation *of Rhizopus* spp.
(Hartati *et al*.,, 2015)

Fermentation

Tempeh production category

Modern process
(fully automated)

Semi modern process

Traditional process
(home industry)

Different fermentation methods and materials (soybean and water) will result in different end product characteristics including metabolome profile

Metabolomics for tempeh studies

Metabolomics approaches was employed to discriminate tempeh based on different production procedure, region of origin, and to relate metabolome profile to quantitative physical characteristics.

Category	Region	
	City	Java
Traditional	Bandung	West
	Bandung	West
	Bogor	West
	Malang	East
	Malang	East
	Malang	East
	Purwokerto	Central
	Purwokerto	Central
	Surabaya	East
	Surabaya	East
	Surabaya	East
	Yogyakarta	Central
Semi Modern	Bogor	West
	Bogor	West
	Yogyakarta	Central
	Yogyakarta	Central
Modern	Surabaya	East

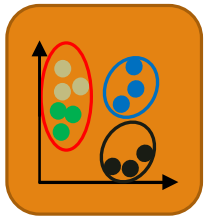
Data Set 1: To reveal effect of **production region**

Data Set 2: To reveal effect of **production procedure category**

→ Metabolome analysis (GC/MS)

Multivariate Data Analysis

PCA (Principal Component Analysis)



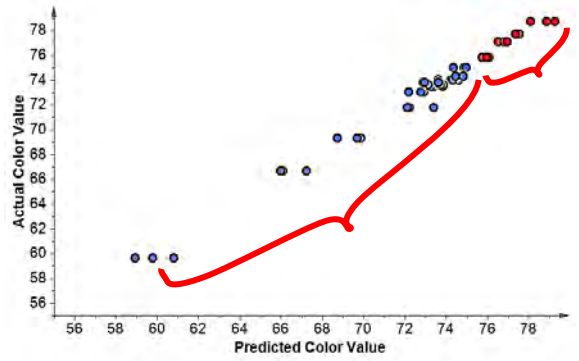
Indonesian tempeh was clustered based on production category

→ Color measurement (Colorimeter CM-2500)

Multivariate Data Analysis

PLS (Projection to Latent Square) Regression Model

Lighter



Darker → **Lighter**

Traditional tempeh generally have darker color compared to semi modern Tempeh

Higher content of 2-aminodipic acid, hydroxybutiric acid, and inositol would give tempeh darker color

Metabolomics based taste evaluation method of white leg shrimp

The present quality evaluation standard of shrimp aquaculture in Japan lacks sensory evaluation method which is important for consumers' acceptance

Shrimp size measurement

Size was utilized as a basis for grading the quality of shrimp

23-32 g/pcs

14-18 g/pcs

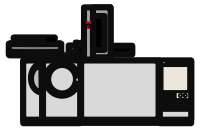
9-11 g/pcs

Sensory evaluation

Semi-trained panelist

Descriptors	Intensity samples		
	A	B	C
Umami	x	x	x
Sweetness	x	x	x
Saltiness	x	x	x

Metabolomics Analysis



Non-targeted metabolomics analysis (GC/MS)

Low molecular compound

Shrimp size-based prediction

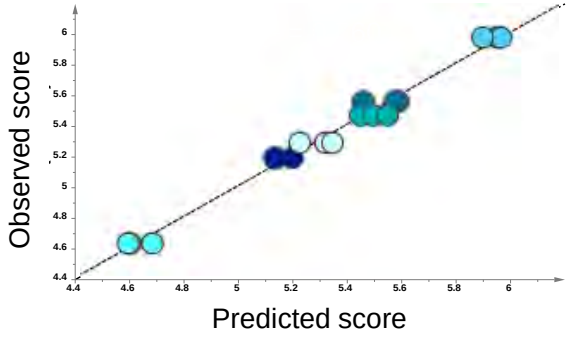
? Could shrimp size predict its taste quality?

There was no linear correlation between shrimp size and its sensory value

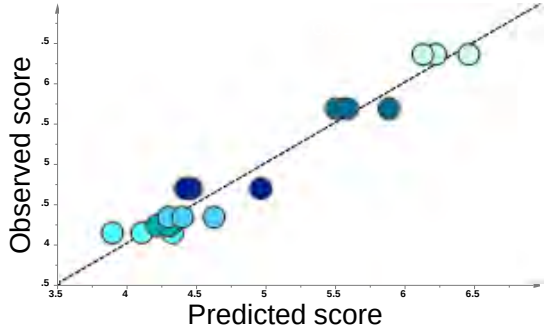
Metabolomics-based prediction

? Could metabolomics predict shrimp taste quality?

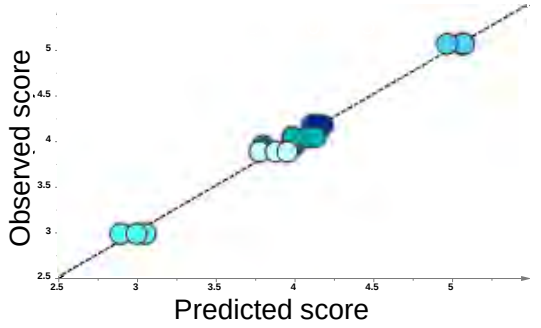
These models indicate that metabolomics was able to predict the sensory value of white leg shrimp



Umami



Sweetness



Saltiness

Metabolomics-based approach for coffee quality

- Coffee is one of the most important commodities traded worldwide. Arabica coffee accounts for majority of the coffee distribution in the world
- Coffee quality is determined mostly based on cupping test by certified panelists

SCAA cupping test

Scored >80 points
out of 100 points

Certified panelists

(SCAA: Specialty Coffee Association of America)

Specialty coffee

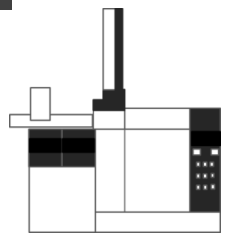
It is important to investigate key compounds which correlate with coffee quality.

Metabolomics is powerful tool to investigate key metabolites for coffee quality

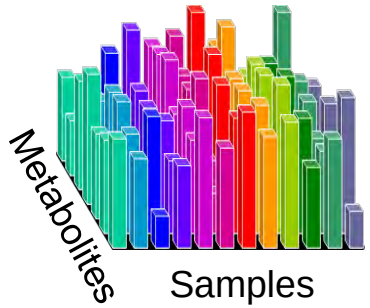
Metabolomics-based approach for coffee quality

Metabolic profiling

Coffee samples
(Green beans)



GC-MS



Sensory evaluation

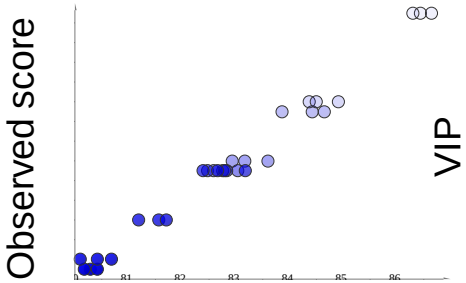
Coffee samples
(Roasted beans)

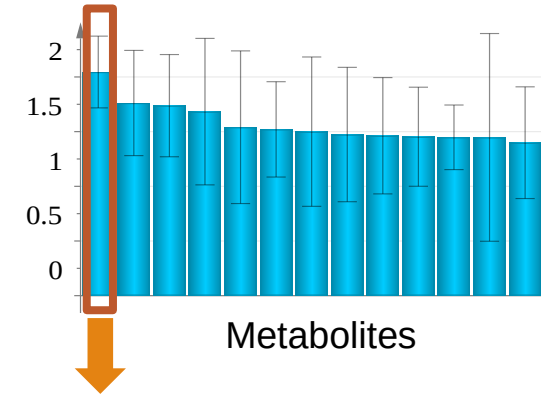
Cupping test

Samples	Cupping score
No.1	81
No.2	84
No.3	78
:	:
No.n	86

Multivariate Data Analysis

OPLS regression analysis





Compound A had the highest VIP value in positive correlating metabolites

Validation

Additional test

Coffee + compound A

VS

Coffee + water (control)

Metabolomics-based research of soy sauce

- Soy sauce has been considered as a major seasoning in east and Southeast Asia
- In recent years, different raw materials of soy sauce has been used to meet the demand

Fermentation process

Koji

LAB

Protein

Amino acids

Enzyme

Starch

Saccharide

Organic acid

Main fermentation yeast

Furanone

・Aroma of caramel

Alcohol

・Enhance of soy sauce aroma

Ester

・Aroma of fruits and flower

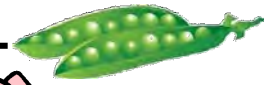
The effect of different raw materials in soy sauce is still unclear

Volatile compound?

Hydrophilic component?

Rice

Green peas



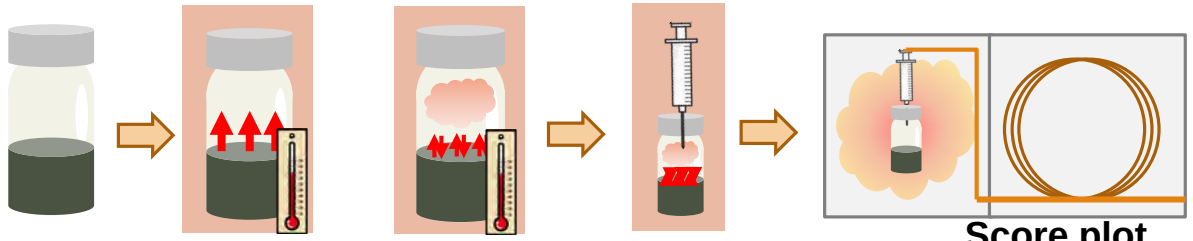
Animal-based

Effect to the flavor?

Metabolomics-based research of soy sauce

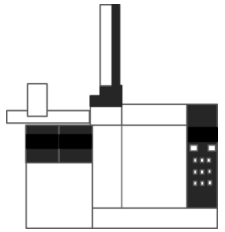
Metabolic profiling

Aroma components



Head Space analysis

Hydrophilic components



GC-MS

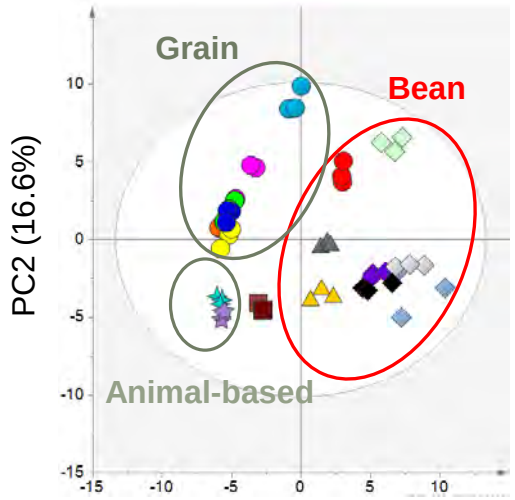
Sensory evaluation

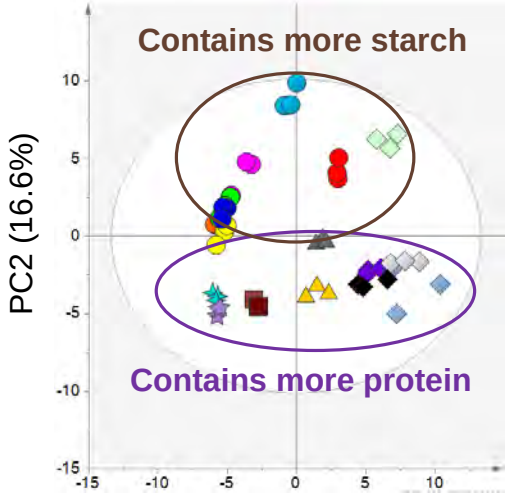
Sensory characteristic

Projective mapping

Multivariate Data Analysis

PCA (Principal Component Analysis)





- According to PC1, the samples were separated between grain, animal-based sample and bean sample
- According to PC2, the samples were separated between metabolites component

Japanese green tea metabolites ranking prediction

- Japanese green tea ranking was obtained based on its sensory score.
- Sensory attributes ranking data was utilized as response variable to predict the quality of Japanese green tea

Japanese green tea

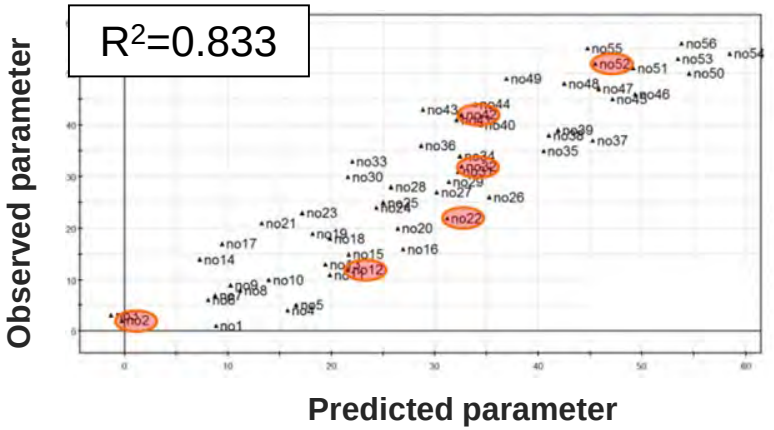
Sensory evaluation

Rank	Green tea
1	Type A
2	Type B
3	Type C
.	.
.	.
64	Type X

Response variable

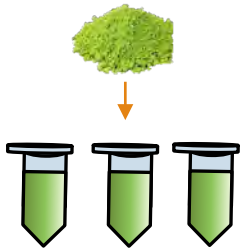
Explanatory variable

Multivariate data analysis



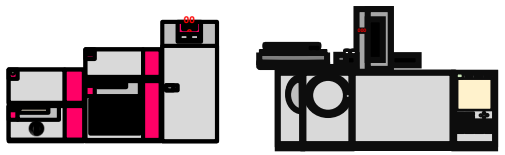
Metabolomics analysis

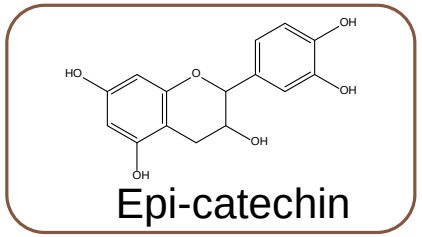
Sample preparation and extraction



Analysis was performed using several instruments

NMR, FT-IR, LC/MS, GC/MS





Epi-catechin

Several high-rank tea has this component as their characteristics metabolites

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)

Soy sauce component profiling analysis

24 soy sauce samples from different origin

Japan	: 13
Chinese	: 6
Amerika	: 3
Other	: 2

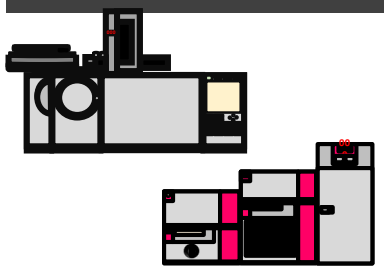
Sensory evaluation

Intensity

Descriptors	samples A	B	C
Umami	x	x	x
Sweetness	x	x	x
Saltiness	x	x	x

Response variable

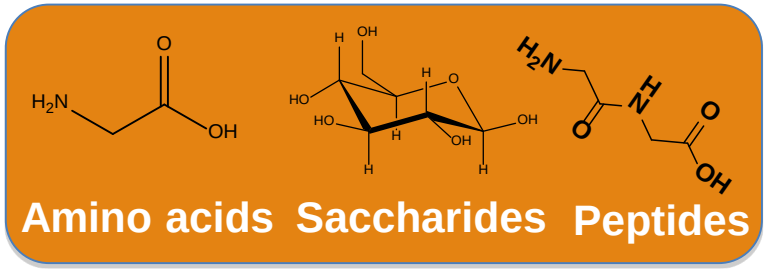
Metabolomics analysis



GC/MS & LC/MS

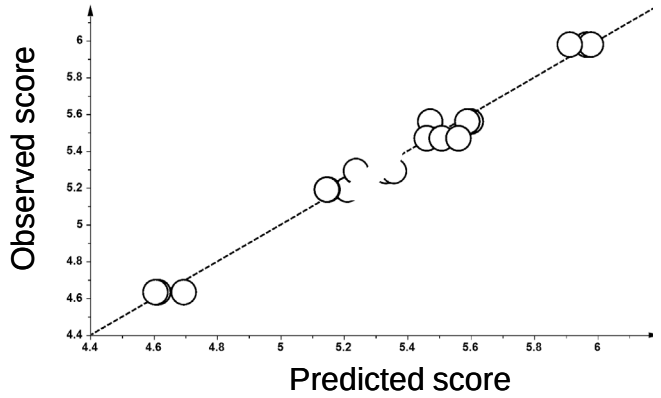
Explanatory variable

Low molecular compounds and peptides



Multivariate Data Analysis

Projection to Latent Structure (PLS)



- Prediction of each attribute intensity from metabolome data
- Identification of highly correlated compound

Yamamoto, *et. al.*, *J. Biosci. Bioeng.*, **114**, 170-175 (2012)
Yamamoto, *et al.*, *J. Biosci. Bioeng.*, **118**, 56-63 (2014)

Japanese green tea-related research published paper

- 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.
- 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)
- 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)
- Miyauchi, S., Yonetani, T., Yuki, T., Tomio, A., Bamba, T., Fukusaki., F., “Quality evaluation of green tea leaf cultured under artificial light condition using gas chromatography/mass spectrometry.”, J. Biosci. Bioeng., 123, 197-203 (2016)

Soy sauce, Fruits, and Luwak Coffee published paper

- 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 114(2):170-175.
- Yamamoto S, Bamba T, Sano A, Kodama Y, Imamura M, Obata A, Fukusaki E. "Analysis of the correlation between dipeptides and taste differences among soy sauces by using metabolomics-based component profiling." *J Biosci Bioeng.* 2014 118(1):56-63
- Shiga K, Yamamoto S, Nakajima A, Kodama Y, Imamura M, Sato T, Uchida R, Obata A, Bamba T,Fukusaki E. "Metabolic Profiling Approach to Explore the Compounds Related to the Umami of Soy Sauce." J Agric Food Chem. 2014 Jul 23;62(29):7317-22. doi: 10.1021/jf501173r. Epub 2014 Jul 11.
- 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.
- Jumhawan 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
- Jumhawan U, Putri SP, Yusianto, Bamba T, Fukusaki E. "Application of gas chromatography/flame ionization detector-based metabolite fingerprinting for authentication of Asian palm civet coffee (Kopi Luwak)." J Biosci Bioeng. 2015 Nov;120(5):555-61. doi: 10.1016/j.jbiosc.2015.03.005. Epub 2015 Apr 23.
- Jumhawan U, Putri SP, Yusianto, Bamba T, Fukusaki E. "Quantification of coffee blends for authentication of Asian palm civet coffee (Kopi Luwak) via metabolomics: A proof of concept." J Biosci Bioeng. 2016 Jul;122(1):79-84. doi: 10.1016/j.jbiosc.2015.12.008. Epub 2016 Jan 6.
- Parijadi AAR, Putri SP, Ridwani S, Dwivany FM, Fukusaki E. "Metabolic profiling of *Garcinia mangostana* (mangosteen) based on ripening stages."J Biosci Bioeng. 2017 Sep 29. pii: S1389-1723(17)30475-9. doi: 10.1016/j.jbiosc.2017.08.013. [Epub ahead of print]
- Yusuke Wada, Atsuki Matsubara, Takato Uchikata, Yugo Iwasaki, Satoshi Morimoto, Katsuta Kan, Tetsuya Ookura, Eiichiro Fukusaki, Takeshi Bamba " Investigation of β-Cryptoxanthin Fatty Acid Ester Compositions in Citrus Fruits Cultivated in Japan" Food and Nutrition 4, 98-104 (2013)

Cheese-related Published paper

- 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)