SHIMADZU
Excellence in Science

Shimadzu
Journal

VOL.
03 ISSUE 2

Food Development and more...

Director's note

Dear Reader,

The year 2015 marks Shimadzu's 140th anniversary, which provides an excellent opportunity to reflect on the past and celebrate our achievements. We recognize that 140 years of continuous operation is the result of the relationships we've built with customers throughout the world who utilize our technologies and solutions to meet their needs. Moving forward, and guided by our corporate philosophy: "Contributing to Society through Science and Technologies" and this year's slogan: "Design the future", we will strive to fulfill customers' wishes more than ever.

Shimadzu Journal has been highlighting various collaborative research projects as well as technical reports and applications from our library since its launch in October 2013. This issue focuses on food development and contains information on recent collaborations. In addition, this issue contains information on other applicable topics, as well as the latest news and applications.

We have two pieces of good news to share in this issue. One is that the new Nexera UC fully automated SFE-SFC-MS system received the Editors' Gold Award at PITTCON 2015 in March. The Nexera UC was developed in collaboration between Shimadzu Corporation, Osaka University, Kobe University and Miyazaki Agricultural Research Institute, which is funded by the Japan Science and Technology Agency (JST).

The other news is the establishment of the new joint research laboratory with Osaka University in April. It is called Osaka University and Shimadzu Analytical Innovation Research Laboratory. This will be used to develop a broad scope of potential applications in such areas as food products, healthcare, pharmaceuticals, chemicals, and the environment using metabolomics. The mentor of this laboratory, Professor Eiichiro Fukusaki from Osaka University and his colleague, Dr. Sastia Prama Putri, appear in this issue and introduce a result of their recent study.

Both examples embody how collaborations with customers can generate innovative instruments and create valuable solutions that deliver true contributions to the world. Please find additional details about each in this issue.

For 140 years, Shimadzu has been developing instrumentation and technology to provide meaningful solutions and exceptional service and support to customers in a variety of markets. We are passionate about the promise the future holds and it's our sincere desire to establish high-quality relationships with you and to exceed your expectations.

Thank you for being a part of Shimadzu's history and future. We hope this journal will be of great help to all of you. We welcome your feedback at any time.

Yours Sincerely,

Teruhisa UEDA, PhD.

General Manager, Analytical & Measurement Instruments Division

CONTENTS

Featuring Food Development

Dr. Sastia Prama Putri and Professor Eiichiro Fukusaki from Osaka University

We interviewed Dr. Sastia Prama Putri, a specially-appointed Assistant Professor in the Department of Biotechnology, Graduate School of Engineering, Osaka University, Japan, and Professor Eiichiro Fukusaki, a leading expert in food metabolomics, about the collaborative research between Osaka University and Indonesian research institute since 2011. One of the significant results was finding out the discriminant marker of the Indonesian Kopi Luwak, which is known as the world's most expensive coffee, but there is no standard method for determining its authenticity. It recently gained worldwide attention and was featured in many mass media including USA Today, BBC UK and so on.

Thank you very much for spending some time for this interview. First, I would like to know about Dr. Putri and your project with the Indonesian government. Could you introduce yourself and tell us about the project including its background, when it has started, what the goal is, your mission and so on?

Putri:

I am originally from Indonesia and I started my career in metabolomics after joining Prof. Fukusaki's lab in 2011. Together with a Ph.D. student from Indonesia, Mr. Udi Jumhawan, we did some brainstorming on the possible research problems that can be solved using the metabolomics approach. We were particularly interested in applying the metabolomics technology to something relevant and unique to Indonesia. Finally, we chose Kopi Luwak because of its high economic value. It is a valuable export commodity for Indonesia, and despite its popularity, very few research groups are working on Kopi Luwak. We realized that one of the most important aspects to pursue in this research project is finding a solid research partner. Therefore, we contacted the Indonesian Coffee and Cocoa Research Institute (ICCRI), one of the leading research institutes and producers of specialty coffee in Indonesia. We identified the most important problem of the Kopi Luwak industry is the lack of a standard method for authentication. This resulted in various attempts of adulteration in the market that damaged the reputation of the industry as a whole. Meanwhile, metabolomics studies have been proven useful for assessing food quality, food safety, food authentication, and determining the origin and varietal differences of food samples. We introduced the concept of metabolic profiling to our colleagues in ICCRI and showed how it can be used to seek for biomarkers that are important for Kopi Luwak authentication. They were very positive about the collaboration and we started the collaborative project in mid-2011. The main purpose of the project was to find biomarkers for Kopi Luwak authentication and establish a low cost and reliable method for routine analysis for Kopi Luwak authentication in Indonesia.

What discoveries or achievements have you made so far?

Putri:

We are very fortunate that through a solid collaboration between Osaka University and researchers in Indonesia, we could identify the discriminant markers for authentication of Kopi Luwak. This research provides a basis for a standardized method for Kopi Luwak authentication. We were also able to discriminate pure Kopi Luwak from those which are blended with other coffee thus, addressing the issue on Kopi Luwak adulteration.

What do you evaluate Shimadzu's instruments? What are our advantages over other vendors?

Fukusaki:

I feel that Shimadzu mass spectrometers have significantly improved in the recent years. In particular, the scan speed of Shimadzu mass spectrometer is superior compared to other vendors. I think that Shimadzu Nexera is the best LC/MS and the price is also quite reasonable.

Putri:

The sales person and after sales support staff are very kind and attentive so I have been very happy with the technical support staff of Shimadzu.

Do you have any requests to us?

Fukusaki:

My request to Shimadzu is the improvement of software for data analysis and the application data, which is not so excellent at this moment. We recently established collaborative laboratory, named Osaka University and Shimadzu Analytical Innovation Laboratory and we hope that this laboratory will contribute to expand and increase the application area. Just a personal opinion, Shimadzu should also put more investment in the instrument design to make it more attractive.

Why did you choose Professor Fukusaki's laboratory as your destination of studying abroad?

Putri:

I was born and raised in Indonesia and moved to Japan in 2004 to pursue my master and PhD studies in the International Center for Biotechnology, Osaka University under Prof. Takuya Nihira's supervision. My PhD work was about structure elucidation of novel bioactive compounds from various natural products and I am also a trained microbiologist. I became interested in the rapidly developing field of metabolomics and joined Prof. Fukusaki's laboratory in January 2011 as a postdoctoral researcher. At that time, there were various metabolomics projects in Fukusaki lab and I was really interested to learn more about the application of metabolomics in various disciplines, including food science, biofuel research and medical applications. Prof.Fukusaki is one of the leading researchers in food metabolomics and he has extensive collaborations with many Japanese companies as well as domestic and international research institutes and universities. Most importantly, his laboratory is very open to international students and it gave me a chance to become a global researcher. In fact, his laboratory is an excellent place for early career researchers such as myself to improve their careers.

Now I would like to ask Professor Fukusaki. I have heard that you have accepted a lot of students from abroad and promote their exchange. What are the benefits?

Fukusaki:

Osaka University aims to establish a global campus, where students can learn in an international surroundings and interact with students coming from many different countries and cultural background. I would like to offer students new opportunities for developing their professional skills in multiple disciplines and communication skills to take active parts in the international scenes. I also believe that the international atmosphere in our laboratory will lead to the expansion of our collaboration network as well as strengthening our leading position in the field of metabolomics.

Do you have anything to add about both the research project and the instruments?

Fukusaki:

One of the most important outcomes of this Kopi Luwak research project is that the method that we develop can be applied as a standard routine procedure for Kopi Luwak authentication. For this purpose, we recently published another paper entitled "Application of GC/FID-based metabolite fingerprinting for authentication of Asian palm civet coffee (Kopi Luwak)". Since GC/FID system is cost effective and is readily available in our research collaborator's institute, we hope that this system can be a practical method for authentication in Kopi Luwak industry.

Could you share your vision of your research and what instrumentation or functions you need to achieve your research goal?

Fukusaki:

Our laboratory's main core is mass spectrometry-based metabolomics, with very wide applications in various fields. We currently have 16 mass spectrometers in our laboratory, including GC/MS, LC/MS, CE/MS, and SFC/MS. In the near future, our laboratory will expand the application area of metabolomics and enrich our toolbox by incorporating NMR technology and imaging mass spectrometry. We are also continuously making efforts to maintain a strong pipeline of collaboration with the private sector as well as domestic and international academic institutions.

It was precious and significant to know what you think of us. Thank you very much.

Here are their recent publications:

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). <http://www.ncbi.nlm.nih.gov/pubmed/25912451> J Biosci Bioeng. 2015 Apr 22. pii: S1389-1723(15)00104-8. doi: 10.1016/j.jbiosc.2015.03.005. [Epub ahead of print]

Mimura N, Isogai A, Iwashita K, Bamba T, Fukusaki E. Gas chromatography/mass spectrometry based component profiling and quality prediction for Japanese sake. <http://www.ncbi.nlm.nih.gov/pubmed/25060729> J Biosci Bioeng. 2014 Oct;118(4):406-14. doi: 10.1016/j.jbiosc.2014.04.006. Epub 2014 Jul 22. Note: this paper won best paper of the year from the Journal of Bioscience and Bioengineering

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. <http://www.ncbi.nlm.nih.gov/pubmed/24954189> Agric Food Chem. 2014 Jul 23;62(29):7317-22. doi: 10.1021/jf501173r. Epub 2014 Jul 11.

Yamamoto S, Shiga K, Kodama Y, Imamura M, Uchida R, Obata A, Bamba T, Fukusaki E. Analysis of the correlation between dipeptides and taste differences among soy sauces by using metabolomics-based component profiling. <http://www.ncbi.nlm.nih.gov/pubmed/24491915> J Biosci Bioeng. 2014 Jul;118(1):56-63. doi: 10.1016/j.jbiosc.2013.12.019. Epub 2014 Feb 1.

Putri SP., Bamba, T., Fukusaki, E. Application of metabolomics for discrimination and sensory predictive modeling of food products. In "Metabolomics - food and nutrition" (e-book). Future Science, London, UK. Dec 2013.

Putri SP and Fukusaki, E. Mass spectrometry-based metabolomics. CRC Press, Taylor and Francis, Boca Raton, USA. 2015

# Application of GC/MS and GC/FID-based metabolomics for authentication of Asian palm civet coffee (Kopi Luwak)

Sastia P. Putri[1], Udi Jumhawan[1] and Eiichiro Fukusaki[1]
[1]Osaka University, Osaka, Japan

Abstract

Kopi Luwak, world's most expensive coffee firstly originated from Indonesia, is made from coffee berries that have been digested by the animal Asian palm civet (*Paradoxurus hemaphroditus*). Despite its profitable prospect, there is no reliable and standardized method for determining its authenticity. Therefore, research on the development of robust method for authentication of Kopi Luwak is urgently needed, particularly to prevent fraud in market worldwide. Twenty-one coffee samples (*Coffea arabica, Coffea canephora*) of three cultivation areas were firstly analyzed by GC-Q/MS. Principal Component Analysis (PCA), Orthogonal Projection to Latent Structures-Discriminant Analysis (OPLS-DA) and Significance Analysis of Microarrays/Metabolites (SAM) of Kopi Luwak was employed to select discrimination marker candidates for authentication of Kopi Luwak. Applicability of discriminant marker candidates was verified using commercial samples. We further established a rapid, reliable and cost-effective analysis employing a universal detector, GC coupled with flame ionization detector (FID) for discrimination analysis of 37 commercial and non-commercial coffee beans extracts. Our study demonstrated that GC/FID-based metabolite fingerprinting can be effectively actualized as an alternative method for coffee authenticity screening in industries.
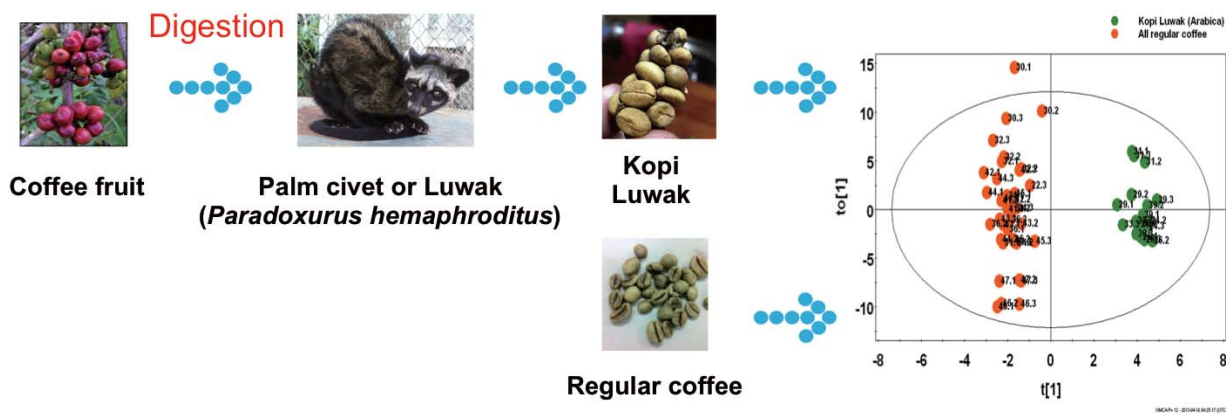
Keywords: Metabolomics; Coffee; Kopi Luwak; Discriminant marker; Authentication; GCMS-QP 2010 Ultra; GC-FID; GC-2010

1. Introduction

The world's most expensive and unique coffee is Kopi Luwak (Kopi is an Indonesian word for coffee and luwak is the animal producing it), one of Indonesia's exotic agricultural products[3]. Marketed with a price tag of 300-400 USD per kg[4], Kopi Luwak's high selling price is attributed to its rarity, unique flavor, and interesting production process. Kopi Luwak is made from coffee cherries that have been eaten by common palm civets (Luwak or *Paradoxurus hemaphroditus*), which use their keen sense of smell to select the best and ripest beans. Kopi Luwak's high quality is mainly contributed by two factors: natural selection of the best coffee cherries by luwak and changes that occur in the digestive track of luwak, which yield an aromatic coffee with flavor described as earthy, syrupy, musty, smooth, and rich with chocolate undertones[5].

Despite its profitable prospect, there is no reliable and standardized method for determining originality of Kopi Luwak. The limited availability of the authentic product and its increasing popularity has opened the possibility of adulteration by blending Kopi Luwak with regular coffee or *in vitro* enzymatic treatment of regular coffee beans to mimic the fermentation by civet in order to increase the production and to meet market demand. This poses serious concern among consumers over the authenticity and the quality of the products currently available in the market. Therefore, research on the development of an unbiased and reproducible method for Kopi Luwak authentication and discrimination is urgently needed, particularly to prevent fraud in Kopi Luwak market worldwide.

Discrimination of Kopi Luwak and regular coffee has been achieved using electronic nose data[5]. However, selection of discriminant marker for authentication was not addressed. The method currently employed by Kopi Luwak producers is by visual and organoleptic testing. Both methods are inadequate because visual examination would only be possible for green coffee bean prior to roasting. As for organoleptic testing, trained experts that could discriminate Kopi Luwak are very few and the test tends to be highly subjective.

Information flows in metabolic pathways are highly dynamic and represent current biological state of individual cells. Hence, metabolome has been considered as the best descriptor of physiological phenomenon[6]. With this capability, metabolomics technique can be a powerful tool to elucidate variations in phenotype imposed by any perturbations such as gene modification, environmental factor, and physical stress. Processes inside animal digestive tract could be translated as physical and enzymatic stress to coffee bean, as it reported to pose smoother surface and color changes after digestion[3]. Thus, metabolomics technique was selected to seek and select discriminant marker for authenticity assessment of Kopi Luwak. Metabolomics technique has been effectively applied to distinguish phytochemical compositions of agricultural products among different origins, varieties, and cultivars for quality control and breeding[7-8].

In this study, gas chromatography coupled with quadrupole mass spectrometry (GC-Q/MS) based metabolic profiling was employed to identify discriminant marker for differentiation of Kopi Luwak and regular coffee. A combination of gas chromatography and mass spectrometry (GC/MS) has demonstrated as an effective analytical platform as it provides high sensitivity, reproducibility and quantitation of large amount of metabolites within a single step extraction[8-9]. Samples classification by means of chemometrics was performed using principal component analysis (PCA). Subsequently, orthogonal projection to latent structures combined with discriminant analysis (OPLS-DA) and significance analysis of microarrays/metabolites (SAM) to identify statistically significant compounds as discriminant marker candidates, was utilized[10-11]. The applicability of discriminant marker candidates was verified to determine authenticity of commercial coffee.

However, a major drawback of this GC-MS-based approach is the high cost of the instrument and maintenance. Therefore, an alternative method is needed for quality and authenticity evaluation of civet coffee. A rapid, reliable and cost-effective analysis employing a universal detector, GC coupled with flame ionization detector (FID), and metabolite fingerprinting has been established for discrimination analysis of 37 commercial and non-commercial coffee beans extracts. GC/FID provided higher sensitivity over a similar range of detected compounds than GC/MS. In combination with multivariate analysis, GC/FID could successfully reproduce quality prediction from GC/MS for differentiation of commercial civet coffee, regular coffee and coffee blend with 50 wt % civet coffee content without prior metabolite details. Our study demonstrated that GC/FID-based metabolite fingerprinting can be effectively actualized as an alternative method for coffee authenticity screening in industries[2].

2. Experimental

Samples and chemicals

Samples were divided into experimental and validation coffee sets. The first set included twenty one coffee beans that were collected from several cultivation areas in Indonesia. Kopi Luwak and regular coffee samples of two species, *Coffea arabica* (Arabica) and *Coffea canephora* (Robusta), were utilized. Coffee samples were obtained from 21 sampling points of three cultivation areas in Indonesia (Java, Sumatera, Bali) as shown in Table 1. In the second experiment for GC-FID application, one Robusta civet coffee sample (Sample no. 17 in Table 1) is removed from analysis resulting in only 20 coffee bean samples. The experimental coffee sets include civet coffee (no. 1-6, Arabica) that had been digested by civet, and undigested beans referred to as regular coffees (no. 7-20, Arabica and Robusta). All coffee samples were treated identically for post harvesting. Coffee samples were roasted in Probat-Werke von Gimborn Maschinenfabrik GmbH model BRZ 2 (Probat, Rhein, Germany) at 205°C for 10 min and followed by immediate air-cooling for 5 min. Roasted coffee beans were kept in sealed Falcon tubes at -30°C until use.
The second set of coffee samples included validation coffee sets. The first validation set consists of authentic Kopi Luwak, commercial Kopi Luwak, commercial regular coffee, imitation/adulteration coffee, and blend coffee (Table 2a). The second validation set consists of 3 civet coffees and 3 regular coffees were bought commercially and 2 additional authentic civet coffees from the Indonesian Coffee and Cocoa Research Institute (Table 2b). In addition, each civet coffee and regular coffee was mixed in equal proportions (50:50, wt %) to obtain representative coffee blends. A total of 17 coffee samples, 8 pure and 9 coffee blends, were then analyzed by GC/MS to verify the established protocol for coffee authentication. All coffee samples were measured in triplicates. All chemicals used in this study were analytical grade.

Extraction and derivatization

To produce fine powder for extraction, coffee beans were ground with a Retsch ball mill (20 Hz, 3 min). Fifteen mg of coffee powder was extracted with 1 mL MeOH/CHCl$_3$/H$_2$O (5/2/2) and added with 60 μL of ribitol (0.2 mg/mL) as internal standard. The samples were centrifuged at 16000 g for 3 min at 4°C. Nine hundred microliter of the supernatant was then transferred into 1.5 mL Eppendorf tube and added with 400 μL Milli-Q water. After re-centrifugation, 400 μL aqueous layer was transferred into a new tube with a pierced cap. The extract was evaporated by vacuum centrifugation for 2 h and freeze-drying overnight. The dried extract was mixed with 100 μL of methoxyamine hydrochloride (20 mg/mL in pyridine) and subsequently incubated at 30°C for 90 min. The second agent, 50 μL MSTFA was added to the mixture and re-incubated at 37°C for 90 min.

Table 1   List of coffee samples (Experimental coffee set)

No.	Origin [a]	Species	Type [b]	Harvest year	Samples extraction [c] period	Samples [d] code
1	Sumatra	*Coffea arabica*	Kopi Luwak	2011	Sept 2011	29
2	Sumatra	*Coffea arabica*	Kopi Luwak	2011	Sept 2011	31
3	Sumatra	*Coffea arabica*	Kopi Luwak	2011	Sept 2011	39
4	Bali	*Coffea arabica*	Kopi Luwak	2011	Sept 2011	33
5	Java	*Coffea arabica*	Kopi Luwak	2011	Sept 2011	24
6	Java	*Coffea arabica*	Kopi Luwak	2011	Sept 2011	26
7	Sumatra	*Coffea arabica*	Regular coffee	2011	Sept 2011	30
8	Sumatra	*Coffea arabica*	Regular coffee	2011	Sept 2011	32
9	Sumatra	*Coffea arabica*	Regular coffee	2011	Sept 2011	40
10	Bali	*Coffea arabica*	Regular coffee	2010	Sept 2011	34
11	Java	*Coffea arabica*	Regular coffee	2011	Sept 2011	22
12	Java	*Coffea arabica*	Regular coffee	2011	Sept 2011	41
13	Java	*Coffea arabica*	Regular coffee	2010	Sept 2011	42
14	Java	*Coffea arabica*	Regular coffee	2011	Sept 2011	45
15	Bali	*Coffea arabica*	Regular coffee	2011	Sept 2011	43
16	Bali	*Coffea arabica*	Regular coffee	2011	Sept 2011	44
17	Sumatra	*Coffea canephora*	Kopi Luwak	2010	Sept 2011	35
18	Sumatra	*Coffea canephora*	Regular coffee	2010	Sept 2011	36
19	Java	*Coffea canephora*	Regular coffee	2011	Sept 2011	46
20	Java	*Coffea canephora*	Regular coffee	2011	Sept 2011	47
21	Java	*Coffea canephora*	Regular coffee	2010	Sept 2011	51

[a] Samples no.1 – 5 and 7 – 11 were originated from same cultivation area
[b] Processing: regular coffee (wet fermentation after harvested), Kopi Luwak (after secreted by animal, washed, and then wet-fermentation)
[c] Samples were obtained from ICCRI during harvest period (around June 2011). Samples from 2010 are ICCRI's coffee collection.
All samples were stored at 4°C and extracted per September 2011
[d] For multivariate analysis

Table 2a   List of coffee samples (Validation coffee set) for the GC/MS-based metabolite profiling to seek for discriminant markers

No.	Brand [a]	Origin	Species	Species	Production year	Samples extraction period
1	Kopi Luwak Andungsari	East Java, Indonesia	*Arabica*	(Authentic) Kopi Luwak	2012	Feb 2013
2	Golden Kopi Luwak	West Java, Indonesia	*Arabica*	(Commercial) Kopi Luwak	2012	Feb 2013
3	Kopi Luwak Wahana	Sumatra, Indonesia	*Arabica*	(Commercial) Kopi Luwak	2012	Feb 2013
4	Kopi Luwak Bali	Bali, Indonesia	*Unknown*	(Commercial) Kopi Luwak	2011	Feb 2013
5	Kopi Wahana	Sumatra, Indonesia	*Arabica*	(Commercial) regular coffee	2012	Feb 2013
6	Kona coffee	Hawaii, USA	*Arabica*	(Commercial) regular coffee	2012	Feb 2013
7	Cerrado Chapado coffee	Brazil	*Arabica*	(Commercial) regular coffee	2012	Feb 2013
8	Aceh Special	Sumatra, Indonesia	*Arabica*	(Commercial) regular coffee	2012	Feb 2013
9	White Koffie	Java, Indonesia	*Arabica*	adulteration coffee	2012	Feb 2013
10	Blend coffee A [b]					
11	Blend coffee B [c]					

[a] Provider 1 ICCRI
2 CV. Kopi Luwak
3 Wahana-Mandheling Kopi
4 Hema-Wiwi Bali
5 Wahana-Mandheling Kopi
6 Hiro coffee
7 Hiro coffee
8 Hiro coffee
9 PT. Java Prima Abadi

[b] Blend between Golden Kopi Luwak (No.2) and Kopi Wahana (No.5) with ratio 50:50 (w/w)

[c] Blend between Kopi Luwak Wahana (No.3) and Kopi Wahana (No.5) with ratio 50:50 (w/w)

Table 2b   List of coffee samples (Validation coffee set) for the GC/FID-based metabolite fingerprinting for validation of method for routine analysis

No.	Brand [a]	Production area	Classification	Production year	Samples ID [b]
1	Andungsari	Java, Indonesia	Civet coffee (authentic)	2012	Au_1
2	Andungsari	Java, Indonesia	Civet coffee (authentic)	2013	Au_2
3	Golden	Java, Indonesia	Civet coffee (commercial)	2012	GO
4	Wahana	Sumatra, Indonesia	Civet coffee (commercial)	2012	WL
5	Bali	Bali, Indonesia	Civet coffee (commercial)	2011	BA
6	Wahana	Sumatra, Indonesia	Regular coffee (commercial)	2012	WR
7	Kona	Hawaii, USA	Regular coffee (commercial)	2012	KO
8	Cerrado Chapado	Brazil, Brazil	Regular coffee (commercial)	2012	CE
9	Coffee blends (9 samples)	Combination of 3 civet coffee (commercial) & 3 regular coffee (commercial)			

[a] Provider 1, 2 ICCRI, Indonesia
3 CV. Kopi Luwak, Indonesia
4, 5 Wahana-Mandheling, Indonesia
6 Hema-Wiwi Bali, Indonesia
7, 8 Hiro coffee, Japan

[b] For multivariate analysis

GC/FID and GC/MS analysis

GC/MS analysis was carried out using GC-Q/MS, GCMS-QP 2010 Ultra (Shimadzu, Kyoto, Japan), installed with CP-SIL 8 CB low bleed column; 0.25 mm × 30 m, 0.25 µm (Varian Inc., Palo Alto, California, USA) and AOC-20i/s autoinjector (Shimadzu, Kyoto, Japan) as an autosampler. Mass spectrometer was tuned and calibrated prior to analysis. A 1 µL of derivatized sample was injected in split mode, 25/1 (v/v), with injection temperature was set to 230°C. The carrier gas flow (Helium) was 1.12 mL/minute with linear velocity, 39 cm/sec. The column temperature was held at 80°C for 2 minute, raised by 15°C/minute to 330°C, and then held for 6 minute. Transfer line and ion source temperature was set to 250°C and 200°C, respectively. A 0.93 kV of electron ionization (EI) was applied to generate ion. Spectra were recorded by 10000 u/sec within mass range 85 - 500 *m/z*. Standard alkane mixture (C_8-C_{40}) was injected at the beginning and end of analysis for tentative identification.

GC/FID was conducted on a GC-2010 (Shimadzu, Kyoto, Japan) installed with an AOC-20s autosampler and AOC-20i autoinjector. One microliter of each derivatized sample was injected in split mode, 25/1 (v/v). To establish proper comparison and validation with the reported GC/MS data, the same type of column, CP-SIL 8 CB low bleed column (0.25 mm × 30 m, 0.25 µm), and identical temperature program were applied to GC/FID analysis. The carrier gas (He) was maintained at a constant velocity of 45 cm/s. The injector and FID temperature were set at 230 and 320°C, respectively.

Data preprocessing

Chromatographic data from GC/MS were converted into ANDI files (Analytical Data Interchange Protocol, *.cdf). This feature include in GC/MS Solution software (Shimadzu, Kyoto, Japan). These ANDI files were subjected to freely available software, MetAlign version 041011, to perform peak detection, baseline correction and peak alignment of retention times[15]. Spectra were normalized manually by adjusting the peak intensity of each sample with internal standard, ribitol. Retention indexes of eluted compounds were calculated based on standard alkane mixture. By comparing its retention indexes and unique mass spectra with in-house reference library constructed from 500 authentic standard chemicals, tentative identification was performed. For comparison with NIST library, retention time was used instead. To simplify and accelerate tentative-identification with compounds that registered in in-house library database, Aloutput2 version 1.29, annotation software developed by authors' laboratory, was utilized[12]. Raw chromatographic data of GC/FID were converted into CDF format using GCMS Solution software package (Shimadzu, Kyoto, Japan). The converted files were subjected to baseline correction, normalization and alignment of retention times using the in-house software, PiroTran ver 1.41 (GL Sciences, Tokyo, Japan). The retention time of internal standard ribitol was confirmed with co-injection of authentic chemical standard before being utilized as reference for normalization and retention time alignment. To reduce the run-to-run variation, the threshold for peak intensity (RSD) was set to < 20%, in each measurement replicate. To construct the data matrix, in which each row and column represent the samples and relative peak intensity at certain retention time, respectively, the outcome data were imported into Pirouette ver 4.0 (Infometrix, Inc, Woodinville, Washington, USA). The data matrix was then subjected to multivariate analysis.

Multivariate data analysis

The details on discriminant marker identification for Kopi Luwak authentication by means of multivariate analyses, namely PCA and OPLS-DA, have been described elsewhere[1]. Briefly, the coffee bean data sets were subjected to supervised discriminant analysis, Orthogonal projection to latent structures-discriminant analysis (OPLS-DA). OPLS-DA was selected to seek and select statistically significant discriminant markers for Kopi Luwak authentication. To confirm selection of significant compounds by OPLS-DA, data were also subjected to MetaboAnalyst 2.0 to perform signifincance analysis of microarrays/metabolites (SAM).

Multivariate analysis was carried out using SIMCA-P+ ver. 13 (Umetrics, Umeå, Sweden) to reduce dimensionality of the huge MS data and extract biological interpretation. PCA and OPLS-DA were used to decipher the relationships between two data matrices, X (predicted variables), and Y (observed variables)[10]. Here, the chromatographic GC/FID data were used as X and for Y, the binary vector of 0 and 1 was assigned for civet coffee and regular coffee, respectively. The data were Pareto scaled prior to analysis without any transformation.

3. Results and Discussion

GC/MS-based metabolite profiling of Kopi Luwak

GC-Q/MS analysis was performed on aqueous extracts of Kopi Luwak and regular coffee bean to investigate the differences in their metabolite profiles to select discriminant marker for robust authentication. Quadrupole mass spectrometer (Q/MS) was selected due to its availability as the most widely used mass analyzer. Therefore, the application of GC/Q-MS is expected to meet with
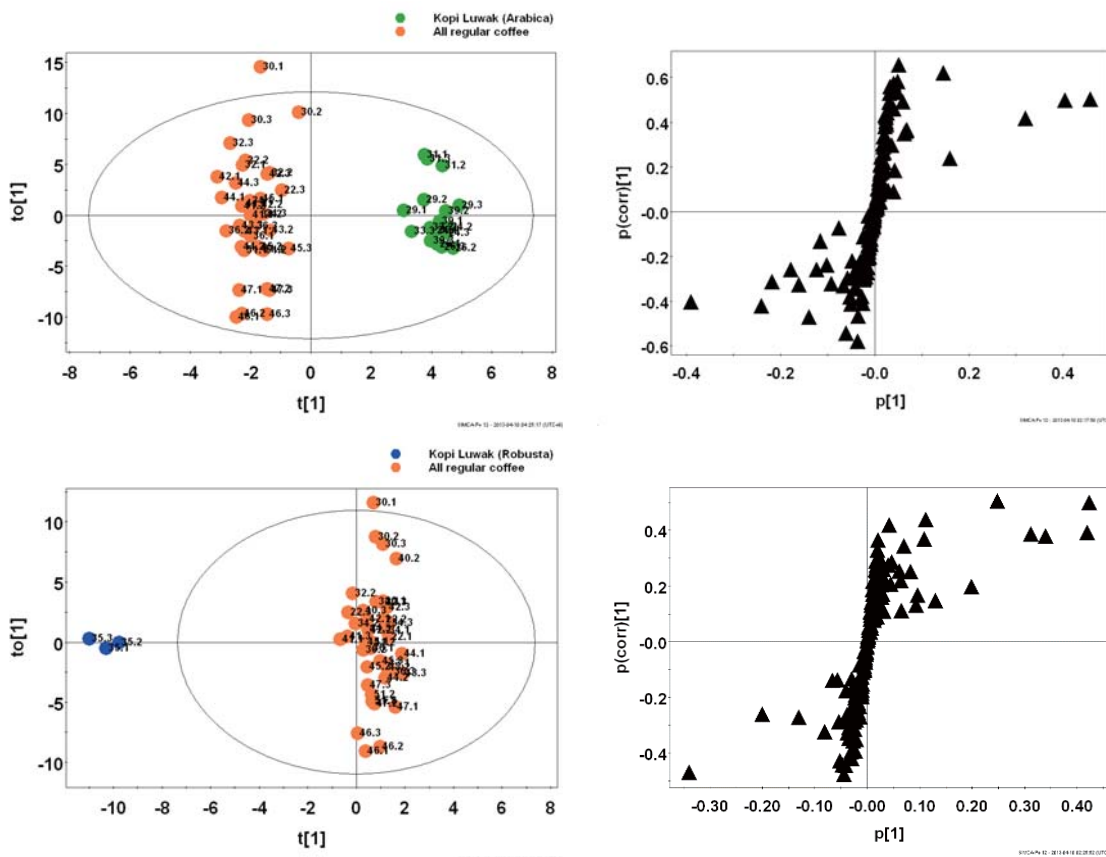

Fig. 1   PCA score plot of Kopi Luwak and regular coffee from same cultivation area, Arabica (right) and Robusta (left).

research objectives. However, the conventional Q/MS can be operated only at slow scan rate[13]. With the improvement of processor and high-speed data processing, newly developed GC-Q/MS provides increased sensitivity in high scan speed up to 10,000 u/sec[14]. Total of 182 peaks from 21 coffee bean samples were extracted using freely available software, MetAlign. Twenty compounds were tentatively identified by comparison with in-house library (retention index) and NIST library (retention time) and six compounds were identified by co-injecting authentic standards. Tentatively identified compounds consist of organic acids, sugars, amino acids, and other compounds. Compounds that have been reported previously in research of coffee bean, including chlorogenic acid, quinic acid, succinic acid, citric acid, and malic acid, caffeine, one of compounds for bitter taste in coffee, and sucrose, the most abundant simple carbohydrates in coffee beans, were identified[1].

To explore an overview of all samples and to obtain general information on sample variances, unsupervised multivariate analysis, PCA, was selected. In previous research, PCA score plot derived from 21 coffee bean displayed differentiation of two data groups based on their species, Arabica and Robusta. Due to large variance between Arabica and Robusta coffee, samples differentiation based on type of coffee (Kopi Luwak and regular coffee) could not be observed. PCA score plot showed data separation based on type of coffee, in which Kopi Luwak (digested by animal) and regular coffee (not digested) can be clearly separated only when the analyses were carried out independently for each coffee species originated from same cultivation area (data not shown).

Discriminant analysis to select candidates of discriminant marker

An overview of all data samples was provided by unsupervised analysis, PCA. However, the detail information regarding contributed compounds for the data differentiation of Kopi Luwak and regular coffee remain unclear. Therefore, coffee bean data sets were subjected to supervised discriminant analysis, OPLS-DA. For analysis

having two or more classes, OPLS-DA is the most suitable analysis platform to isolate and select differentiation marker. The analysis provides visualization of the covariance and correlation between compounds and the constructed discrimination model. Compounds that are highly contribute to the model and their reliability may possess potentially biochemically interesting characteristic, thus it can be selected as biomarker candidates[10]. The S-plot featured in OPLS-DA visualizes covariance (contribution or magnitude) and correlation (reliability) between metabolites and modelled clas[7]. Potential candidates of discriminant markers for authenticity assessment of Kopi Luwak can be selected via S-plot by setting the cut off for covariance, $p[1]$, and correlation value, $p[corr]$, $> |0.2|$. S-plot of coffee data sets was shown in Fig. 1 In addition to cut off value, compounds for candidates of discriminant markers were selected by its VIP (variable importance in projection) value. Large VIP (> 1) values are more relevant for model construction.

OPLS-DA score plot of Arabica coffee data sets were shown in Fig. 1A. Discrimination between Kopi Luwak and regular coffee was obtained. The model explained the goodness-of-fit parameter (R^2) and the predictability parameter (Q^2), 0.965 and 0.892, respectively (0.936 and 0.829 after cross validation). This model was considered good based on the above criteria. Significant compounds for samples separation were plotted at the top and bottom of S-plot. Interestingly, compounds uncorrelated with Kopi Luwak were quinic acid, caffeine and caffeic acid. As for predictive compounds (component to correlate with Kopi Luwak) in above cut-off value, consist of citric acid, malic acid and glycolic acid. OPLS-DA score plot of Robusta coffee data sets (Fig. 1B) were explained by R^2 and Q^2, 0.957 and 0.818, respectively (0.957 and 0.833 after cross validation). Inositol and caffeine (p, p[corr] exceeded cut-off value), were selected as discriminant marker candidates from OPLS-DA.

We employed significance analysis of microarrays/metabolites (SAM) to select significant compounds for discriminant marker as comparison to OPLS-DA. SAM indicates a total of 12 compounds considered as significant in Arabica coffee data set (Fig. 2). Of those compounds,
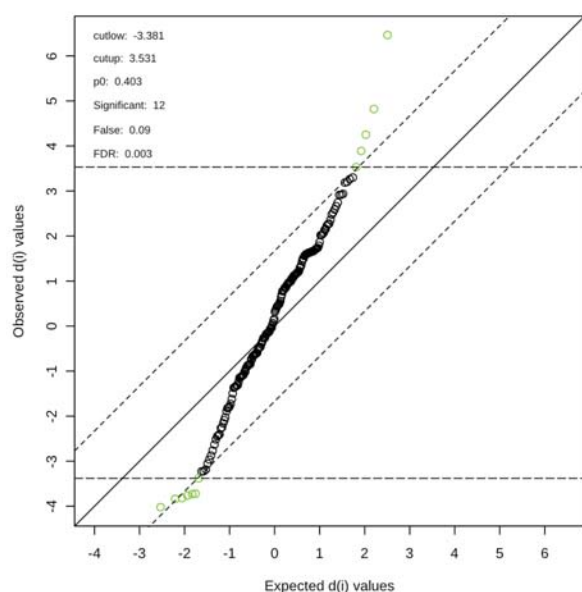
Fig. 2   SAM analysis derived from Kopi Luwak (Arabica) and all regular coffee data sets of experimental coffee set. Twelve detected peaks (highlighted by green color) were considered significant. The list including glycolic acid, malic acid, citric acid, quinic acid and unidentified peaks.

Table 3   Tentative identification

Name	Retention time (tR) in minute	RSD of tR (n=3)	Identification [a] method
Pyruvate	4.69	0.094	a
Lactic acid	4.79	0.086	a, b
Glycolic acid	4.99	0.079	a, b, c
Oxalate	5.62	0.074	a
Succinic acid	7.54	0.615	a, b
Glyceric acid	7.65	0.051	a, b
Fumaric acid	7.89	0.051	a, b
Malic acid	9.09	0.039	a, b, c
Aspartic acid	9.37	2.16	a, b
Pyroglutamic acid	9.47	0.04	a, b, c
Pyrogallol	9.59	0.041	a
Arabinose	10.47	0.035	a, b
Arabitol	10.9	0.034	a, b
Shikimic acid	11.57	0.032	a
Citric acid	11.68	0.031	a, b, c
Quinic acid	11.93	0.032	a
Fructose	12	0.031	a, b
Glucose	12.15	0.041	a, b
Caffeine	12.18	0.038	a, b, c
Inositol	13.5	0.028	a, b, c
Caffeic acid	13.85	0.025	a, b
Tryptophan	14.36	0.602	a, b
Octadecanoic acid	14.49	0.025	a, b
Sucrose	16.49	0.023	a, b
Melibiose	17.33	0.025	a, b
Chlorogenic acid	18.76	0.025	a

[a] (a = in-house library, b = NIST library, c = authentic standard)

citric acid, glycolic acid, and malic acid, identified from OPLS-DA, and other unidentified peaks were included. In Robusta coffee data set, 9 significant compounds were found. The result showed that inositol, caffeine and pyroglutamic acid and six unidentified peaks possessed high significance in SAM[1]. Candidates of discriminant marker for authentication assessment for Arabica and Robusta coffee were listed in Table 3. Marker candidates were selected for those met with significant criteria in both OPLS-DA and SAM. Discriminant markers were then selected independently for Arabica and Robusta coffee due to great variances among coffee species.

Validation of the applicability of discriminant marker for authenticity assessment

To verify the applicability of selected marker candidates, we have conducted analysis of validation set including authentic Kopi Luwak, commercial Kopi Luwak, commercial regular coffee, adulteration coffee, and blend coffee. Processing of authentic Kopi Luwak was controlled comprehensively. To provide unbiased analysis, the rest of samples were purchased commercially. In general, from harvest to pre-roasting, samples labelled as "commercial Kopi Luwak" and "commercial regular coffee", were processed in similar way to produce Kopi Luwak and regular coffee in the experimental set, respectively. However during roasting, their respective providers often to apply different parameter. Imitation coffee was processed to reduce its acidity in order to obtain characteristic close to Kopi Luwak[4]. Commercial regular coffees were selected from different cultivation area. To examine feasibility the selected marker to differentiate pure and blend coffee, we mixed two commercial Kopi Luwak, Golden Kopi Luwak and Kopi Luwak Wahana, and commercial regular coffee (Kopi Wahana) with ratio 50:50 (w/w), respectively, to compare applicability of discriminant marker to perform when blending was carried out by coffee beans from same and different cultivation area.

By employing all detected peaks to PCA, samples were populated into four clusters with the largest variance correspond to imitation coffee as it clearly separated from others (data not shown). Next, we projected six marker candidates as inclusion list into PCA to obtain overview of applicability of marker candidates for samples differentiation. Similar to prior strategy, separation of those four groups coffee was observed. The PCA was explained by 59.5% and 20.9% variance in PC1 and PC2, respectively (Fig. 3). Imitation coffee was populated separately by PC1. Separation was likely due to attempt by producer in order to obtain close characteristic of Kopi Luwak. In PC2, commercial Kopi Luwak, blend coffee, and commercial regular coffee could be differentiated. Both authentic and commercial Kopi Luwak was clustered within near area. In spite of originated from different country and processed with different parameter, commercial regular coffee were clustered in near region, suggesting these factors have least significance for data separation. From the loading plot information, citric acid, malic acid and inositol exhibited high contribution value for Kopi Luwak data sets. Interestingly, these three marker candidates also showed highest VIP value for constructing discriminant model (Table 1).

To display the applicability of selected discriminant markers to differentiate samples in validation set, box plot was constructed using relative peak intensity of citric acid, malic acid and inositol. Box plot of malic acid and citric acid were able to differentiate commercial Kopi Luwak (Kopi Luwak Wahana), blend coffee, commercial regular coffee (Kopi Wahana) and adulteration coffee. However, box plot of inositol failed to differentiate these samples. Hence, we selected double marker ratio, inositol/pyroglutamic acid (Fig. 4). Pyroglutamic acid was selected for having lowest contribution for separation of Kopi Luwak and regular coffee. We confirmed ratio of blend coffee by quantitate the constituent of discriminant marker. Analytical parameter for quantitation was shown in Table 3. All authentic standards exhibited good linearity of 0.99 or more for at least seven points in the applied concentration range. To examine the validity of quantitation, limit of detection (LOD) and limit of quantitation for each discriminant marker were also determined. The amount of six discriminant marker candidates in coffee sample was quantitated higher than the LOD and LOQ of authentic standards. Concentration of selected marker (malic acid, citric acid, and inositol/pyroglutamic acid) in all blend samples was in range of 48.5 ± 0.02 to 52.3 ± 0.75% (Fig. 4). The result corresponded well with box plot of peak intensity for each discriminant marker. We confirmed feasibility of the proposed strategy for robust authentication of Kopi Luwak in pure and blend coffee for ratio of 50:50.
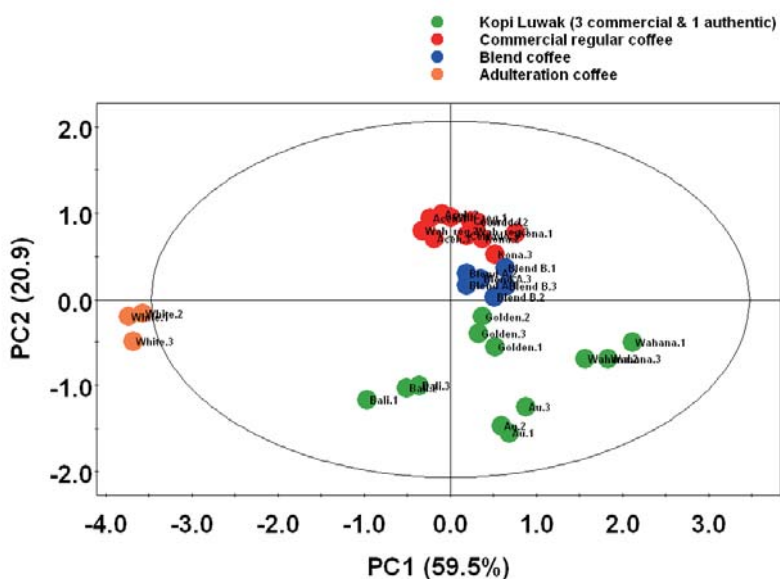
Fig. 3   PCA score plot of validation coffee set. Separation with adulteration coffee was obtained in PC1, while commercial Kopi Luwak, blend coffee and commercial regular coffee can be differentiated in PC2

Malic acid

Citric acid

Inositol/Pyroglutamic acid
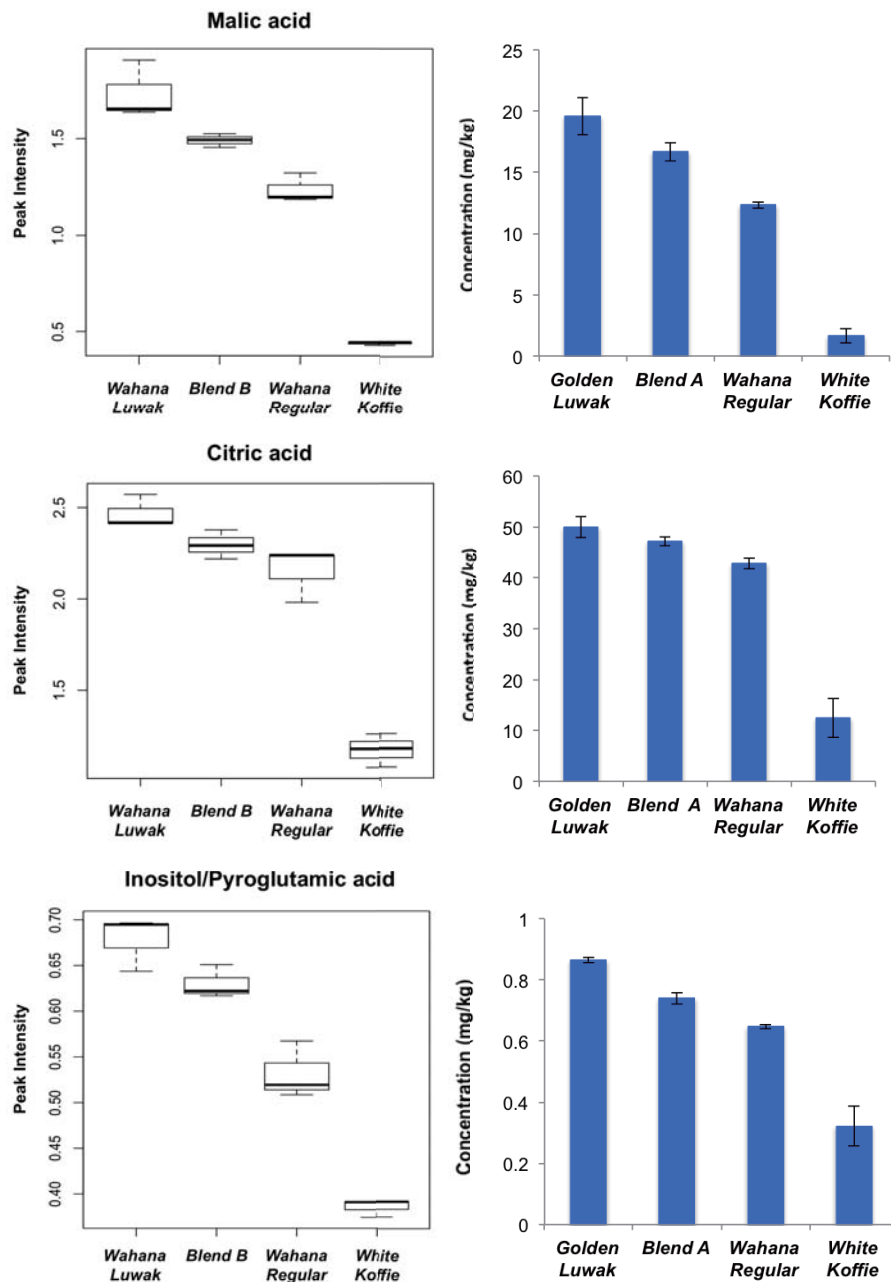
Fig. 4   Box plots of peak intensity and concentration for selected discriminant markers of validation coffee sets (for Blend coffee A)
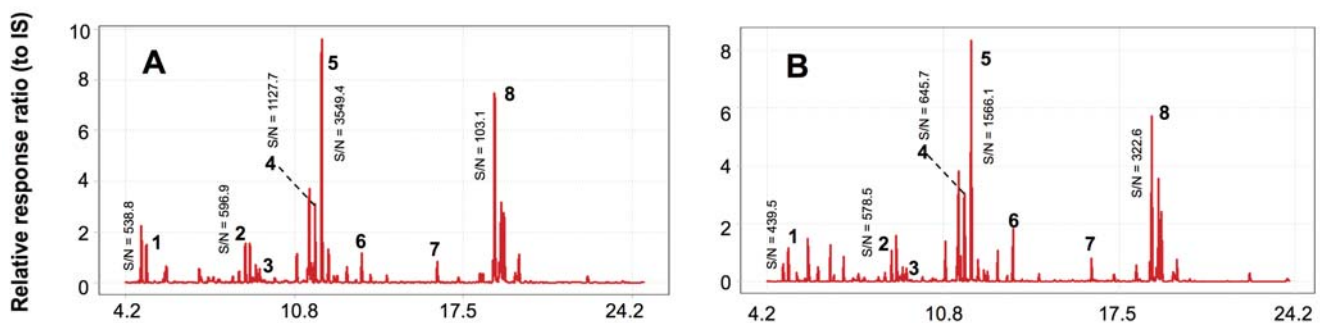
Fig. 5   Gas chromatograms of representative coffee bean extracts obtained from (A) GC/FID; and (B) GC/MS analysis. Both analyses used same column, CP-SIL 8 CB low bleed. Peak tentative identification: (1) glycolic acid, (2) malic acid, (3) pyroglutamic acid, (4) citric acid, (5) quinic acid, (6) inositol, (7) sucrose, and (8) chlorogenic acid. S/N, signal-to-noise ratio.
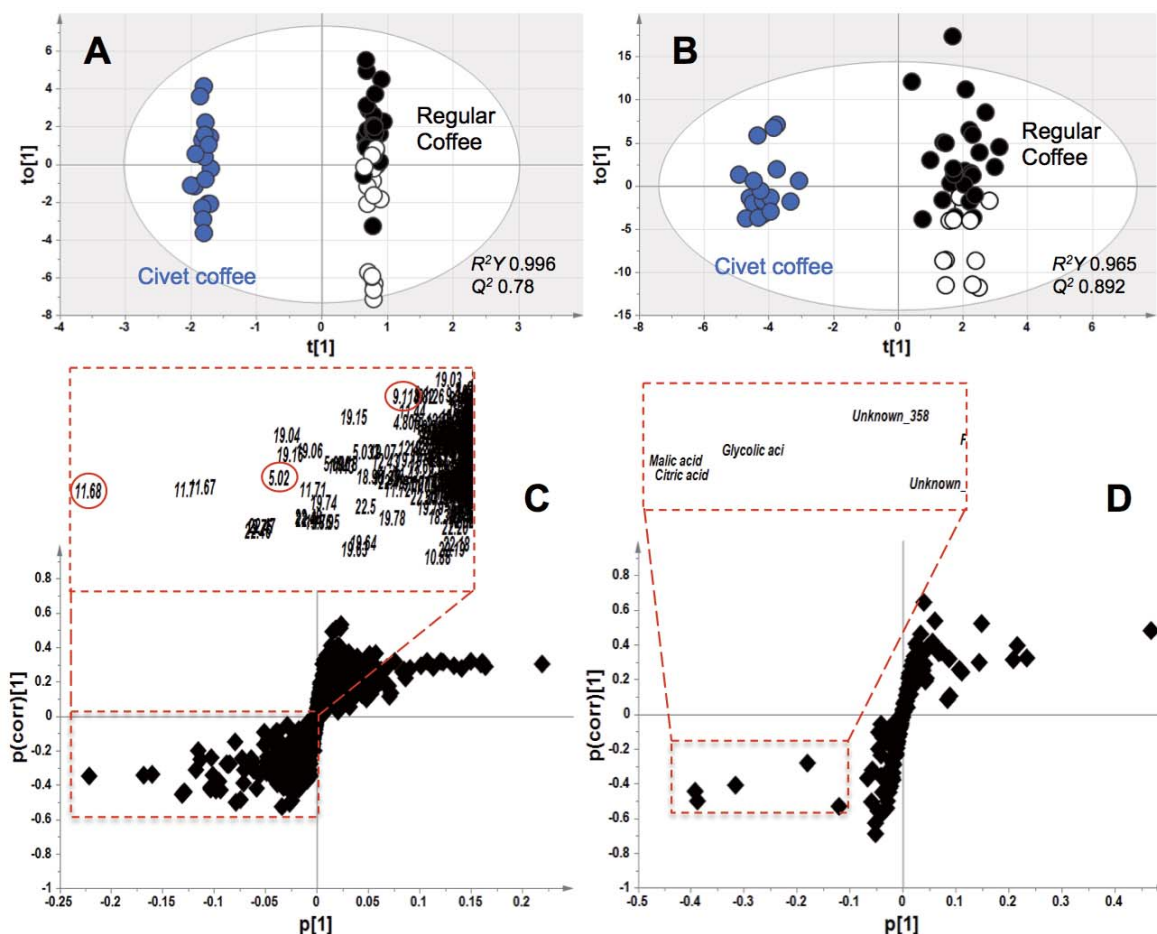
Fig. 6 OPLS-DA score plots and S-plots based on (A,C) GC/FID and (B, D) GC/MS chromatograms of 20 coffee bean extracts. The S-plot displayed the covariance *p* against correlation *p*(corr) of the variables to the model class designation. The closed diamonds represent each variable (detected peak) used for model construction; identities of variables with high reliability to civet coffee are given in the inset figure.

GC/FID-based metabolite fingerprinting for Kopi Luwak authentication

We compared the chromatogram obtained from GC/FID with the one from GC/MS analysis reported previously (2) using the same coffee extract and column type. The chromatographic data of GC/FID and GC/MS gave similar metabolite patterns as shown in Fig. 5, which contained the peaks from diverse metabolites, i.e. glycolic acid (peak no. 1), malic acid (peak no. 2), pyroglutamic acid (peak no. 3), citric acid, (peak no. 4) quinic acid (peak no. 5), inositol (peak no. 6), sucrose (peak no. 7) and chlorogenic acid (peak no. 8). A total of 678 peaks were obtained from GC/FID, compared to 182 peaks from GC/MS analysis.

For metabolite fingerprinting, it is not necessary to determine the individual information of every peak[16]. Nonetheless to confirm the overall quality of GC/FID analysis, peak confirmation of the GC/FID chromatogram was performed by comparing to the identified peaks in the GC/MS data and co-injection of authentic chemical standards. Whilst most of the detected peaks that represented key coffee metabolites were identical between GC/FID and GC/MS, we also observed a shift in their retention times, such as in glycolic acid (5.02 and 4.96 min), malic acid (9.11 and 9.05 min), and citric acid (11.68 and 11.61 min), respectively. Since metabolomics

data are often subject to unwanted variations[17], the retention time shift reported here, albeit not severe, may be due to experimental variation between analytical instruments.

Although the overall chromatographic profiles between GC/FID and GC/MS were similar, it is noticeable that GC/FID analysis provided higher relative peak intensity than GC/MS for almost all detected peaks. The higher relative peak intensity often implies higher sensitivity as GC/FID analysis has been described to generate higher sensitivity compared to the mass detector which frequently operated in a full-scan mode for gathering entire profiles of biological sample[2]. Measurement of total ions over mass range resulted in the limitation of sensitivity for the mass detector. The efficient reduction of relative intensity for detected peaks within the range of 4.2 and 6 min was also observable for GC/FID analysis. The peaks were confirmed by comparison with the NIST library and identified as siloxane, common peak contaminants from injector and vial septa. The result was explicable since FID primarily responds to a wide variety of carbon-containing organic compounds whereas a mass detector relies on the recognition of the entire ionized and fragmented

molecules. The results suggested the practicability of using GC/FID for metabolite fingerprinting of coffee beans as it provided higher sensitivity over a similar range of detected compounds than GC/MS analysis.

We carried out a comparison of the multivariate analyses obtained from GC/MS analysis with that of GC/FID in order to evaluate the performance quality of the latter platform[2]. Based on the results of multivariate analyses, we could confirm the practicability of the GC/FID coupled to metabolite fingerprinting strategy for rapid discrimination and prediction of new samples with statistical significance (Fig. 6).

Finally, a set of commercial samples from the coffee market has been analyzed to provide scientific evidence of the GC/FID application in the coffee industry. Since processing commercial samples is based on the customers' preference, the roasting temperature may vary from experimental coffee. Commercially available regular and civet coffee were selected from different production areas. To set the validation threshold, we acquired two authentic civet coffees from different production years as benchmark samples. Furthermore, a total of 9 coffee blends were prepared from the combination of each commercial sample with mixing ratio of 50:50 (wt %). These four differentiation parameters, occurrence of perturbation, production area, roasting parameter and mixing ratio, would present comprehensive coverage for validation. The validation experiment was successful to verify the feasibility of employing the significant variables obtained from GC/FID for practical use in authentication of Kopi Luwak.

Conclusion

Our findings highlighted the utility of metabolic profiling using GC/MS combined with multivariate analysis for selection of discriminant marker for authenticity assessment of valuable agricultural products. Once the discriminant markers have been identified, we propose the utility of GC/FID coupled with metabolite fingerprinting as a good complementary and cost effective analysis platform for quality assessment of civet coffee. The GC/FID system offered high-speed analysis for coffee quality assessment. This advantage can be beneficial to manufacturers for quality control, especially for authentication of commercial coffee and other agricultural products in industrial scale.

References

1 Jumhawan, U.; Putri, S. P.; Yusianto, Marwani, E; Bamba, T.; and Fukusaki, E. Selection of Discriminant Markers for Authentication of Asian Palm Civet Coffee (Kopi Luwak): A Metabolomics Approach, J. *Agric. Food Chem*. **2013**, *61*, 7994–8001.

2 Jumhawan, U.; Putri, S. P.; Yusianto, Bamba, T.; and Fukusaki, E. Application of GC/FID-based metabolite fingerprinting for authentication of Asian palm civet coffee (Kopi Luwak), *J. Biosci. Bioeng*. **2015**. Available online 23 Apr 2015.

3
http://www.nytimes.com/2010/04/18/world/asia/18civetcoffee.html (Last access: April 3, 2013)

4 Joshi, A. R.; Smith, J. L. D.; Cuthbert, F. Influence of food distribution and predation pressure on spacing behavior in palm civets. *J. Mammal*. **1995**, *76*, 1205-1212.

5 Marcone, M. F. Composition and properties of Indonesian palm civet coffee (Kopi Luwak) and Ethiopian civet coffee. *Food Res Int*. **2004**, *37*, 901–912.

6 Blow, N. Metabolomics: biochemistry's new look. *Nature*. **2008**, *455*, 697-700.

7 Wei, F.; Furihata, K.; Koda, M.; Hu, F.; Kato, R.; Miyakawa, T.; Tanokura, M. ^{13}C NMR-based metabolomics for the classification of green coffee beans according to variety and origin. *J. Agric. Food Chem*. **2012**, *60*, 10118-10125.

8 Pongsuwan, W.; Fukusaki, E.; Bamba, T.; Yonetani, T.; Yamahara, T.; Kobayashi, A. Prediction of Japanese green tea ranking by gas chromatography/mass spectrometry-based hydrophilic metabolite profiling. *J. Agric. Food Chem*. **2007**, *55*, 231-236.

9 Jonsson, P.; Johansson, E. S.; Wuolikainen, A.; Lindberg, J.; Schuppe-Koistinen, I.; Kusano, M.; Sjostrom, M.; Trygg, J.; Moritz, T.; Antti, H. Predictive metabolite profiling applying hierarchical multivariate curve resolution to GC-MS data — a potential tool for multi-parametric diagnosis. *J. Prot. Res*. **2006**, 5, 1407–1414.

10 Wiklund, S.; Johansson, E.; Sjostrom, L.; Mellerowicz, E. J.; Edlund, U.; Shockcor, J. P.; Gottfries, J.; Moritz, T.; Trygg, J. Visualization of GC/TOF-MS-based metabolomics data for identification of biochemically interesting compounds using OPLS class models. *Anal. Chem*. **2008**, *80*, 115–122.

11 Tusher, V.G.; Tibshirani, R.; Chu, G. Significance analysis of microarrays applied to the ionizing radiation response. *Proc. Natl Acad. Sci. USA*. **2001**, *98*, 5116-5121.

12 Tsugawa, H.; Tsujimoto, Y.; Arita, M.; Bamba, T.; Fukusaki, E. GC/MS based metabolomics: development of a data mining system for metabolite identification by using soft independent modeling of class analogy (SIMCA). *BMC Bioinf*. **2011**, *12*, 131–143.

13 Dalluge, J.; Vreuis, R. J. J., van Iperen, D. J.; van Rijn, M.; Brinkman, U. A. T. Resistively heated gas chromatography coupled to quadrupole mass spectrometry. *J. Sep. Sci*. **2002**, *25*, 608-614.

14 http://www.shimadzu.com/an/gcms/qpultra.html (Last access: May 7, 2015)

15 Wermelinger, T.; D'Ambrosio, L.; Klopproge, B.; Yeretzian, C. Quantification of the robusta fraction in a coffee blend via raman spectroscopy: proof of principle. *J. Agric. Food Chem*. **2011**, *59*, 9074-9079.

16 Fiehn, O.; Metabolomics--the link between genotypes and phenotypes, Plant Mol. Biol. **2002**, 155–71

17 De Livera, A. M.; Dias, D. A.; De Souzza, D., Rupasinghe, T.; Pyke, J.; Tull, D.; Roessner, U.; McConville, M.; and Speed, T. P.; Normalizing and integrating metabolomics data, *Anal. Chem*. **2012**, *84*, 10768–76.

Development of GC-MS analysis methods for essential oil distributed nonuniformly in foods

Takehiko Sagawa, Ph.D, Research and Analysis Center, Research and Product Development Divsion, Development and Manufacuturing Group, S&B Foods Inc.

Abstract

The quality control of aroma in spices and herbs is essential for the development of food products in companies dealing with spices and herbs. Product development in food companies involves quality control; it is also important for providing information about spices and herbs, which are required in several recipes or for marketing purposes to explain their significance in cooking. In this study, we focus on essential oils localized in foods, which affect their flavor. Furthermore, the role and possibility of chemical analysis for improving good flavor are examined.

Introduction

A flavor influencing the gusto of the foods is constructed in "aroma" and "taste". Recently, the importance of the effects of aroma on gusto has been attracting significant attention. Hence, odor-active compounds in foods have been examined by chemical analysis because the understanding and control of food aroma are essential for the development of food products. A previous study utilizes a different approach involving the sensory analysis of foods using uniform and non-uniform model food products containing odor-active compounds. However, with this approach, food product developers could not correlate the results obtained from chemical analyses with those obtained from the sensory evaluations of foods. One way to solve this issue is to use homogeneous analytical samples for chemical analysis. However, food products are complex as well as non-homogeneous from both physical and chemical viewpoints. Herein, we report two examples for the measurement of odor-active compounds localized in foods. The first example describes the measurement of an aroma compound in herbs involving the migration of thymol from fresh thyme to cod fish in the case of "foil-roasted cod fish with fresh thyme". The second example describes the measurement of an odor-active compound (E)-2-dodecenal localized in coriander leaves and roots. In both cases, direct sample introduction-gas chromatography–mass spectrometry (DMI-GC/MS) analysis was employed.

The DMI device reported by Amirav et al.[1,2] performed well in the analysis of a non-purified sample with direct introduction of the sample. The device has a component with a programmed temperature vaporizing (PTV) injector and a specialized glass liner with space for a microvial holding the solid sample.

Several studies using the DMI device have reported the analysis of pesticide residues and the combination with the quick, easy, cheap, effective, and rugged method, a known simple purification method.[3,4] One case described a method in which nonvolatile compounds were kept in a specific microvial during analysis to avoid instrument and capillary GC column contamination,[5] in which volatile compounds were drained into the split vent before starting GC/MS analysis for cleanup in a glass liner.[6] In another case, screening analysis for polycyclic aromatic hydrocarbons was performed using a DSI device.[7] A small amount of atmospheric aerosols was collected directly into a glass liner for analysis. The efficacy of this method for microchemical screening analysis was also reported in this study.

DMI-GC/MS has been used with plant tissue to evaluate the quality of herbal medicine with chemical fingerprinting[38] and to analyze methyl salicylate in plants infected with the tobacco mosaic virus.[39] A DMI device allows various introductions for GC analysis. In this study, we developed a DMI-GC/MS analysis technique for extraction of volatile compounds from small tissues of a food and analysis of the quality of the essential oil in foods.

This analytical method permits the GC/MS measurement of volatile compounds using milligram amounts of sample.

1. Thyme flavor in the case of foil-roasted cod fish with fresh thyme
– Measurement of thymol localized in the case of prepared cod fish –

We employed DMI-GC/MS for analyzing the aroma of prepared foods. For example, we confirmed the migration of thymol from thyme to a slice of cod fish in foil-roasted cod fish with fresh thyme.

Sample preparation

Fig. 1 shows the preparation of DMI-GC/MS analysis of the cod fish tissues. The tissue section was inserted into the specialized microvial (30 μL) carefully to avoid breakage (Fig. 1). The vial was then set in the glass liner of the Optic4 (DMI liner, ATAS GL International B.V.), following which GC/MS analysis was performed.

We subjected the samples thus prepared to DMI-GC/MS analysis to investigate the migration of thymol from a micro viewpoint.
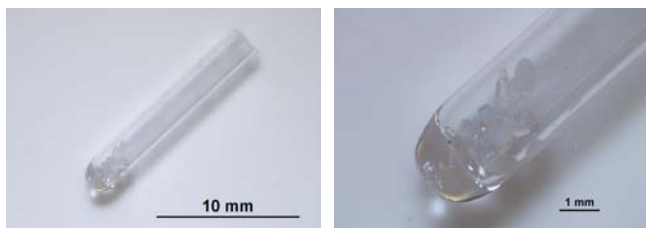
Fig. 1 A piece of cod fish tissues in the specialized micro vial for DMI-GC/MS analysis

GC/MS analysis

GC/MS spectra were recorded on a Shimadzu QP2010 Ultra system using a Stabilwax fused-silica capillary column (60 m × 0.25 mm × 0.25 μm film thickness; Restec, Pennsylvania, USA). Oven temperature, 60°C for 2 min, then increased to 100°C at a ramp rate of 10°C/min, maintained constant at 100°C for 1 min, increased to 200°C at a ramp rate of 5°C/min, and finally increased to 230°C at a ramp rate of 7°C/min; transfer line temperature, 230°C; carrier gas, helium with a linear velocity of 30 cm/s; split mode (10:1); ionization energy, 70 eV; scan time, 500 ms; mass range, 45 - 300 amu. DMI conditions: sample amount, 2 mg.

 PTV injector conditions: the initial temperature of 50°C was increased to 120°C for 2 min at a ramp rate of 10°C/s. Cryofocus, −80°C for 2 min and then increased to 150°C at a ramp rate of 30°C/s.

Results and discussion

As shown in Fig. 2-1, three thyme leaves were placed on a cod fish slice and roasted in a foil. Two samples were collected from this roasted cod fish slice and subjected to DMI-GC/MS analysis: one from the area covered by the thyme leaf and the other from the area that was not covered by the thyme leaf (Figs. 2-2, 3, and 4). Thymol was detected only from the sample collected from under the leaf and not from the sample collected near the leaf (Figs. 2-5, 6), thus confirming the migration of thymol from thyme to the fish slice from a micro viewpoint. Then, we roasted a slice of the cod fish with fresh thyme and performed DMI-GC/MS analysis at three points (Figs. 3-1 and 2). Each total ion current chromatogram (TICC) showed that the amounts of thymol migrated at the three points were different and that the migration of thymol into the fish slice was not uniform (Figs. 3-3, 4, and 5). These results demonstrated the practical applicability of DMI-GC/MS analysis. The distribution of specific aroma compounds in cooked foods is considered to be non-uniform, and the difference in the physicochemical properties of cooking foods possibly affects the aroma released during their consumption. Hence, various approaches employed in this study are expected to complement the sensory evaluation of cooking foods.
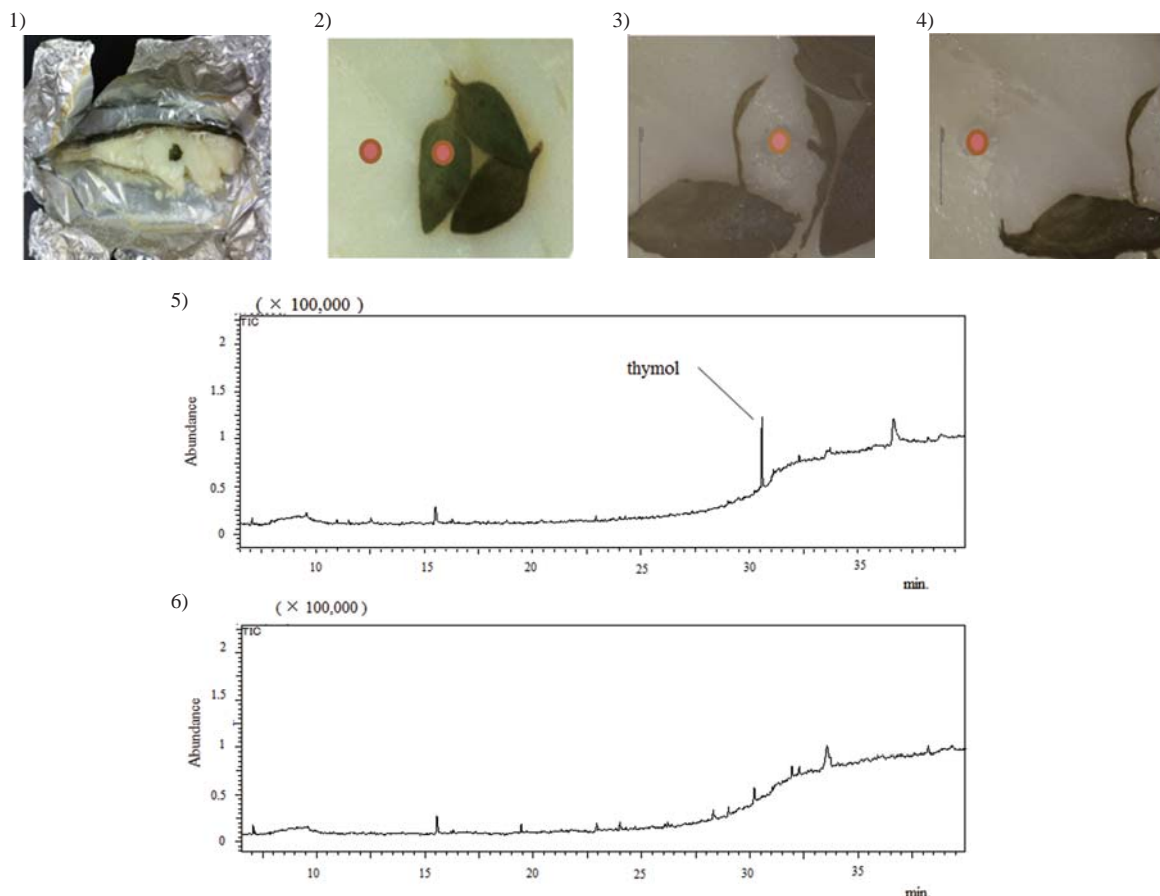
Fig. 2 Migration of thymol from a fresh thyme leaf to a cod fish slice

1) Image of a cod fish slice with three thyme leaves after roasting in a foil; 2) magnified image focusing on thyme leaves, where circles denote the sampled area; 3) sampled point under a thyme leaf; 4) sampled point near a thyme leaf; 5) TICC of the sampled point under the thyme leaf obtained by DMI-GC/MS analysis; 6) TICC of the sampled point near the thyme leaf obtained by DMI-GC/MS analysis.
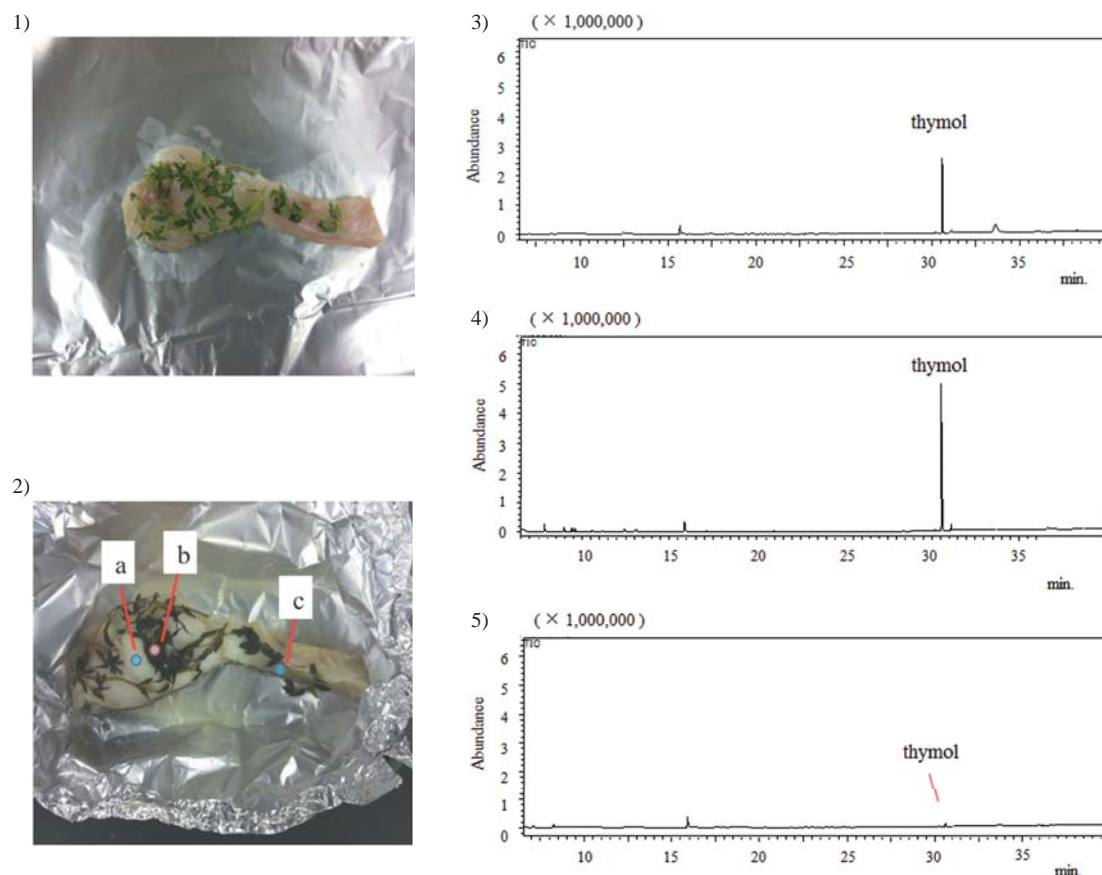
Fig. 3 Migration of thymol in the case of foil-roasted cod fish with fresh thyme

1) Image of cod fish with fresh thyme leaves before roasting in a foil. 2) Image of foil-roasted cod fish with fresh thyme leaves. a, b, and c denote the sampled points for DMI-GC/MS analysis. 3), 4), and 5) show TICCs of the sampled points a, b, and c, respectively. For DMI-GC/MS analysis, the amount of each sample was 2 mg.

2. The odor-active compounds localized in coriander leaves and roots
– An odor-active compound *(E)*-2-dodecenal localized in vines –

Coriander plants are known to contain two major odor-active compounds: *(E)*-2-dodecenal and *(E)*-2-tetradecenal.[8,9] In particular, *(E)*-2-dodecenal has a strong odor. Coriander plants are commonly used in salads: Leaves are used in salads, while their roots are used as a flavor dressing. We confirmed the odor-active compounds localized in a leaf and a root. Furthermore, we employed DMI-GC/MS to analyze the aroma of prepared foods, e.g., the localization of the aroma compounds in coriander leaves and roots.

Sample preparation

Fig. 4 shows the sample preparation of the coriander leaves for DMI-GC/MS analysis. The sample preparation was the same as those employed for the DMI-GC/MS measurement of foil-roasted cod fish with thyme.
Furthermore, we employed DMI-GC/MS to analyze the localization of the aroma compound from a micro viewpoint.

GC/MS analysis

The GC/MS conditions were the same as those employed for the DMI-GC/MS measurement of foil-roasted cod fish with thyme.

Results and discussion

Coriander leaves and roots were subjected to DMI-GC/MS analyses. The results indicated that the vines of the coriander leaf contain a majority of *(E)*-2-dodecenal (Figs. 5 and 6). This result suggests that *(E)*-2-dodecenal is possibly distributed non-uniformly in the coriander leaf, thereby imparting a good taste. On the other hand, the surfaces of the coriander root contain both *(E)*-2-dodecenal and *(E)*-2-tetradecenal (Fig. 7). Hence, coriander roots are possibly better for use as a flavor dressing, because the odor-active compounds of the coriander roots migrate into the dressing effectively.
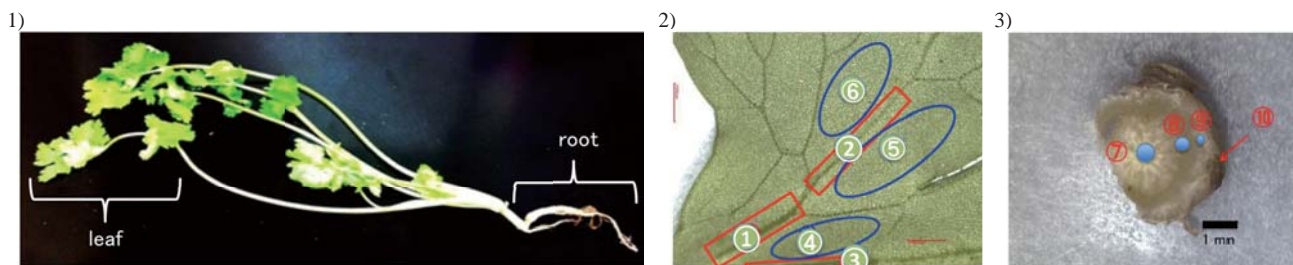
Fig. 4 Sampling area for DMI-GC/MS analysis of the coriander leaf and root

1) A plant of coriander. 2) Magnified view of the coriander leaf. Red rectangles denote vines, while blue ellipses denote the spaces between the vines. 3) Magnified view of a coriander root slice. Blue circles (⑦ – ⑨) and ⑩ have been targeted. The area indicated by the red arrow ⑩denotes the surface of the coriander root. All areas are cut off, and each piece is used for DMI-GC/MS analysis, similar to the prepared sample Fig. 1.
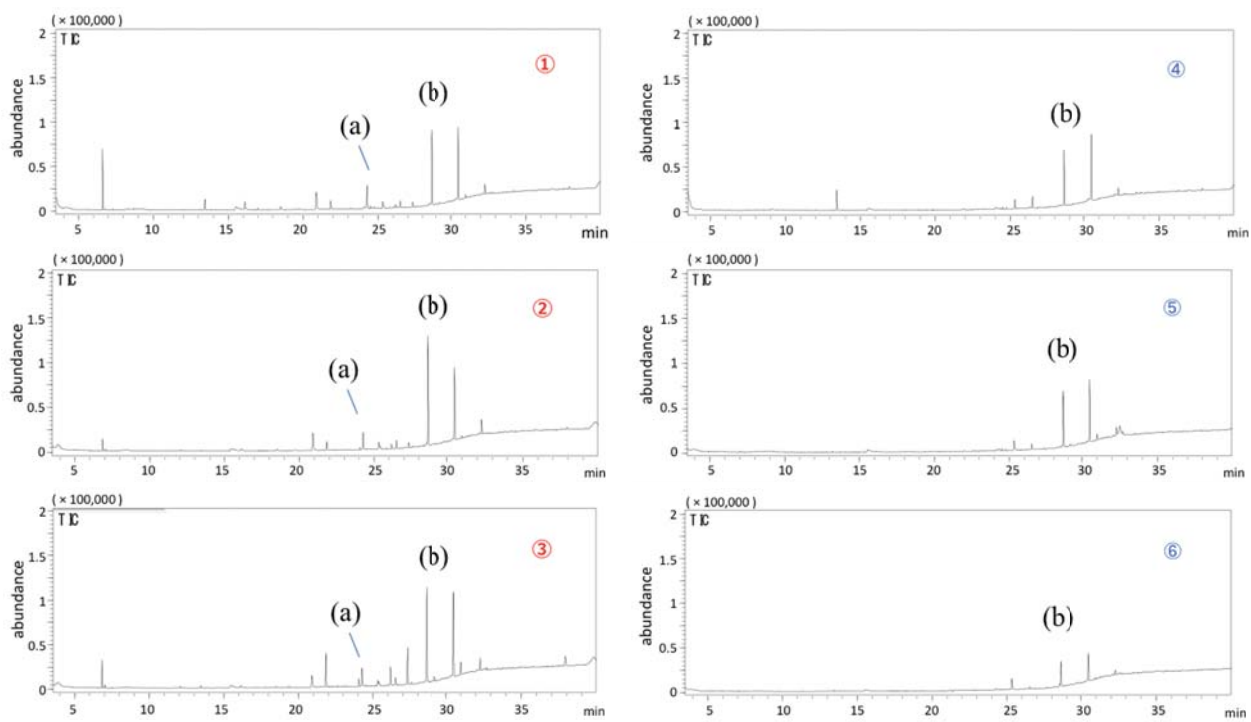
Fig. 5 Total ion current chromatograms of the coriander leaf obtained from DMI-GC/MS analysis

TICC of samples (1–6) obtained from DMI-GC/MS analysis. Samples 1–3 denote the TICC of the vines of coriander leaf. Samples 4–6 denote the TICC of the spaces between the vines of the coriander leaf. a: *(E)*-2-dodecenal. b): *(E)*-2-tetradecenal.
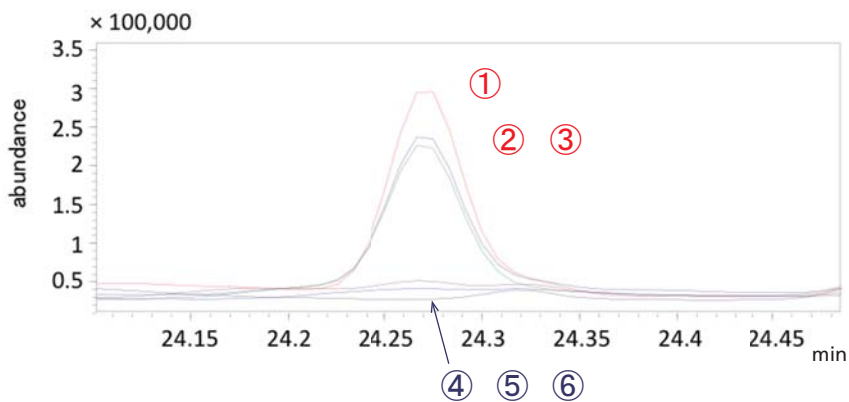
Fig. 6 Magnified view of the TICC showing the retention time of *(E)*-2-dodecenal

Samples 1–3 denote the TICC of the vines of the coriander leaf. Samples 4–6 denote the TICC of the spaces between the vines of the coriander leaf.
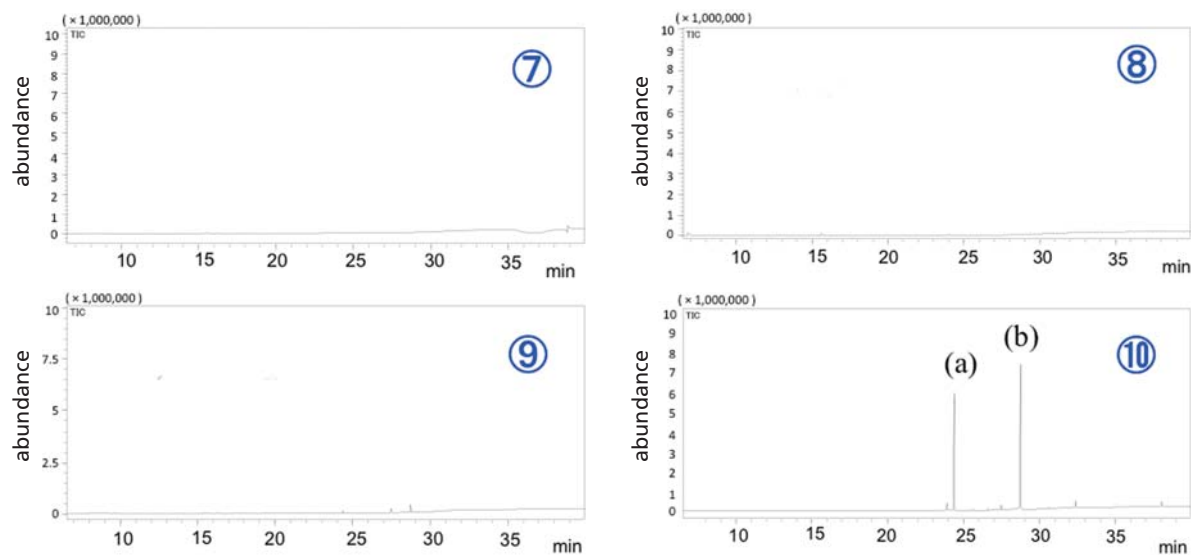
Fig. 7 TICC of a sample piece of the coriander root obtained from DMI-GC/MS analysis; sample numbers are the same as those in Fig. 4-2

Samples 7–10 denote the same samples shown in Fig. 4-2. a: *(E)*-2-dodecenal. b): *(E)*-2-tetradecenal.

Conclusions

The chemical analysis of volatile compounds in foods is generally performed under the condition of averaged samples. However, we cannot perceive gusto with homogenized foods, and foods are non-homogeneous from a micro viewpoint.
Hence, chemical analysis can be employed for measuring volatile compounds localized in foods for understanding the gusto of foods.

References

[1] Amirav A., Dagan S., Eur. Mass Spectrom., 3, 105-111 (1997).

[2] Jing H., Amirav A., Anal. Chem., 69, 1426-1435 (1997).

[3] Čajka T., Maštovská T., Lehotay S. J., Hajšlová J., J. Sep. Sci., 28, 1048-1060 (2005).

[4] De Koning S., Lach G., Linkerhägner M., Löscher R., Tablack P. H., Brinkman U. A. T., J. Chromatogr. A., 1008, 247-25 (2003).

[5] Cunha S. C., Lehotay S. J., Mastovska K., Fernandes J. O., Beatriz M., Oliveira P. P., J. Sep. Sci., 30, 620-632 (2007).

[6] Ng C. I., Zhang X., Talnta 85, 1766-1771 (2011).

[7] Falkovich A. H., Rudich Y., Environ. Sci. Technol., 35, 2326-2333 (2001).

[8] Quynh C.T.T., Kubota K. *Food Sci. Technol. Res*. 18(2), 287-294 (2012)

[9] Eyres G., Dufour J. P., Hallifax G., Sotheeswaran S., Marriott P. *J. Sep. Sci*. 28,1061-1074 (2005)

Shimadzu Selection

These articles were selected by Shimadzu. Relating to food analysis, they are from application notes and technical reports. They feature a variety of instruments we produce and include cutting-edge technologies. Please obtain the articles of your interest through the links on the titles.

Selection 1   Food Analysis

FTIR-ATR Characterization of Commercial Honey Samples and Their Adulteration with Sugar Syrups Using Chemometric Analysis

Honey is a valuable food commodity that gains a lot of attention due to its potential health benefits and usage to sweeten foods and beverages. To save on manufacturing costs, honey is susceptible to deliberate adulteration with sugar syrups such as corn syrup. FTIR with attenuated total reflectance (ATR) was used to collect absorption spectra of various commercial honey samples.

Selection 2   Food Analysis

In Wine There is Truth - The Characterisation and Quantitative Analysis of Wine Using Spectroscopic Methods

Due to globalisation, food is no longer a local product, but may be transported over thousands of kilometres from its source to where it is consumed. Unfortunately, the additives for preservation as well as the packaging material are potential sources of contamination. From the very beginning, Shimadzu has been involved in the development of analytical methods related to European regulations and food guidelines. One recent example is the European wine regulation. This paper shows the Shimadzu spectroscopic methods for residue analysis and quality control of wine.

* Reprinted with the kind permission of International Labmate Ltd - first printed in International Labmate Buyers Guide 2014/15 www.intlabmate.com

Selection 3   Food Analysis

Analysis of Sugars and Sugar Alcohols in Energy Drink by Prominence-i with Differential Refractive Index Detector

Sugars and sugar alcohols display almost no ultraviolet absorption, and are therefore typically detected using a differential refractive index detector or evaporative light scattering detector. This note presents an example of simultaneous analysis of sugars and sugar alcohols in an energy drink using the Prominence-i and RID-20A.

Selection 4   Food Analysis

Simultaneous Analysis of Water- and Fat-Soluble Vitamins in Beverages Using an ODS-Modified Metal-Doped Column

In this study, a Shim-pack MAqC-ODS I was used to analyze water- and fat-soluble vitamins in a single method. This column contains ODS-modified and metal-doped stationary phase, so it was able to retain the weakly-retained components without ion-pairing reagents.

Selection 5   Food Analysis

Quantitative Analysis of Anabolic Steroids in Control Samples from Food-producing Animals using a Column-switching LC-HESI-MS/MS Assay

The use of natural and synthetic hormones to increase the weight of meat-producing animals is prohibited in the European Union. This paper shows the development of an online-SPE method to considerably shorten the pre-treatment time. In addition to optimisation of the extraction method, chromatographic separation was optimised to decrease ion-suppression and isobaric interference.

Selection 6   Food Analysis

Quantitative Analysis of Veterinary Drugs Using the Shimadzu LCMS-8050 Triple Quadrupole Mass Spectrometer

Foods in which chemical residues, like pesticides, feed additives, and veterinary drugs, found in excess of maximum residue levels have been banned from sale in many countries around the world. This Application News introduces an example of the high-sensitivity analysis of 89 veterinary drugs in a crude extract of livestock and fishery products.

Selection 7 | **Food Analysis**

Analysis of Ethylene in Food Using GC/MS

Ethylene is a type of plant hormone. It promotes the growth of fruits and vegetables, and suppresses the sprouting of potatoes. This experiment details the analysis of ethylene produced by apples.

Selection 8 | **Food Analysis**

Using Headspace SPME-GC×GC–quad MS for the Characterization of Marsala Wine

This contribution describes a research work focused on the elucidation of the composition of the headspace of Marsala wine. Samples were subjected to headspace solid-phase microextraction-comprehensive 2D GC analysis. At the outlet of the second GC dimension, the eluting analytes were split between a flame ionisation detector (for relative quantification purposes) and a rapid-scanning quadrupole mass spectrometer (for compound identification). The results attained open a door on the highly complex nature of the Marsala headspace.

Selection 9 | **Food Analysis**

Comprehensive 2D GC with Dual Mass Spectrometry / Flame Ionization Detection for the Analysis of the Unsaponifiable Fraction of Vegetable Oils

A comprehensive two-dimensional GC (GC×GC) method, with dual FID/MS detection was developed for the qualitative and quantitative analysis of the entire unsaponifiable fraction of vegetable oils. The analysis of the fatty acid (FA) composition of a vegetable oil can provide valuable information on both its quality and genuineness. However, because many oils have a similar FA profile, the investigation of a minor but highly specific group of compounds, namely the unsaponifiable fraction, is helpful for the assessment of a possible adulteration.

Selection 10 | **Food Analysis**

Comprehensive 2D GC with Dual Mass Spectrometry / Flame Ionization Detection for the Analysis of the Milk Unsaponifiable Lipid Fraction

A comprehensive two-dimensional GC (GC×GC) method, with dual FID/MS detection, was developed for the qualitative and quantitative analysis of the entire unsaponifiable fraction of lipids belonging to various milk-types. The GC×GC column set used consisted of a low-polarity first dimension and a medium-polarity secondary one, both characterized by a high thermal stability. The use of dual detection enabled the simultaneous attainment of both qualitative (mass spectral information) and quantitative data (%).

Selection 11 | **Food Analysis**

Novel Comprehensive Two-dimensional LC and Related Application for Complex Samples

A novel two-dimensional LC (2D LC) has been developed and successfully applied to phospholipids, polyphenols and medicinal compounds in biological, food and crude drug samples, respectively.

Selection 12 | **Food Analysis**

Qualitative Determination of Genetically Modified Ingredients in Soybean and Corn Using the MultiNA and the PCR Method

Genomes in three groups of soybean and corn samples were extracted using a plant genome extraction reagent kit. MultiNA was used to determine if any genetically modified ingredients were present. The results indicate that no genetically modified ingredients were detected in the three groups of soybean samples, while the NOS exogenous gene was detected in two groups of corn samples.

Selection 13 | **Food Analysis**

Characterization of the Lactobacillus Casei Group Based on Profiling of Ribosomal Proteins Coded in S10-spc-alpha Operons as Observed by MALDI-TOF MS

The taxonomy of the members of the Lactobacillus casei group is complicated due to the genetic similarities within the group and controversial nomenclature status. We were able to conduct quick and accurate discrimination of the L. casei group using as markers ribosomal proteins detected during measurement of bacteria by MALDI-TOF MS.

Flavor release evaluation kit can be used as a tool in food development for evaluating flavors released during chewing

by Robert H. Clifford, PhD, and Satoshi Yamaki, PhD

Food knows no boundaries. As people immigrate around the world they bring the culture and favorite recipes with them. Food companies try to duplicate these recipes but need to offer some advantages whether it is meal preparation time, cooking time, cost or some combination of these. The problem is sometimes the recipes aren't quite right as the texture and flavors are slightly off.

The perceived taste of food is related to both the flavor of the food and as well as the texture. The human mouth is able to ascertain and describe food texture in terms of hardness, adhesiveness, cohesiveness, brittleness, elasticity, gumminess, and chewiness. These parameters can be defined using a mechanical testing machine as shown in Fig. 1. At the same time, the flavors of food released during the chewing process are detected subjectively by the nose. Each discrete "chew" may release different types of aromatic flavors as well as different concentrations, depending on the type of food.

The mouth can only detect 5 unique tastes which are sweet, sour, salt, bitter and umami (savory); however it has been estimate the nose can detector anywhere from 10,000 to a trillion different odors. Thus in recent years much attention in research and development has turned to evaluation of the favors released during chewing.

A new tool has been developed to evaluate the subjective texture of a food product, and simultaneously collect aromatic compounds that are released during chewing shown in Fig. 2 by the "EZ Test Release Evaluation Kit". The evaluation tool seals the sample, the sample holder, and plunger in a dedicated sample bag. It is able to perform the texture evaluation test in the sealed sample bag, and capture the released flavor and aroma compounds for subsequent analysis during the texture testing process.

Flavor-release measurements can be made before and after each texture test. The amount and composition of the flavors and aromas released by each "chew", or series of "chews", can then be compared. The flavor compounds are collected by solid-phase micro-extraction (SPME). The SPME unit is then inserted into a heated injection port of a GCMS where the flavors are released and analyzed.

Texture and flavor are important factors in the taste of foods. In this application a piece of an apple was evaluated for the flavor released during the "chewing" process. The results of flavor analysis of the apple by GCMS are shown in Fig. 3. The gray chromatogram shows the flavor analysis before the texture test, and the red chromatogram is after the texture test. By comparing the gray and red chromatograms, it can be seen that some peaks increased in intensity and some peaks remained the same. The

- Hardness (H)
- Brittleness (B)
- Adhesiveness (A3)
- Cohesiveness (A2/A1)
- Elasticity (T2/T1)
- Rubberness (H × A2/A1)
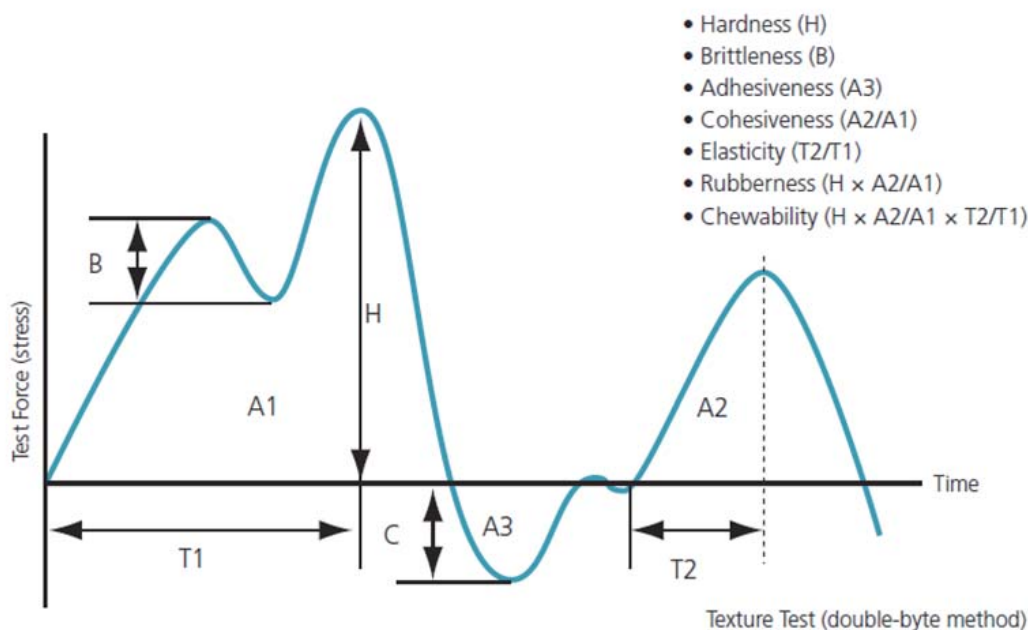- Chewability (H × A2/A1 × T2/T1)

Fig. 1 Texture Analysis Parameters by Universal Testing Machine

peaks which increased in intensity are marked with a green circles above them. Therefore, some flavors were released by destroying or "chewing" the apple sarcocarp during the texture test. The increased peaks were determined to be alcohols and esters by a mass spectrum library search.

The texture component the apple was tested for was brittleness. Mechanical characteristic curve of the apple by texture test are shown in Fig. 4. During the texture test 5 different "chews" of the apple were tested in different sampling positions shown from the 5 peaks. The first peak expresses the texture characteristics. The chromatograms in Fig. 3 show the cumulative aromas released during the 5 "chews".

Table 1 shows the compounds from the library search and the percent increase from the "chewing" process. The compounds released with the percent increase in concentration in parentheses are: 1) 2-Methylbutyl acetate (96%), 2) sec-Butylcarbinol (16%), 3) Ethyl hexanoate (108%), 4) n-Hexyl acetate (130%),5) n-Hexanol (43%), 6) n-Hexyl butyrate (202%), 7) Hexyl 2-methylbutyrate (192%), 8) 6-Methyl-5-hepten-2-ol (58%), and 9) Farnesene (264%).

Looking at the first example of these compounds, the 2-Methylbutyl acetate is listed as a food and flavor ingredient according to Sigma-Aldrich and can be purchased as an additive to foods. The second compound sec-Butylcarbinol can be produced during the distillation of alcohol and can be grouped with the fusel oil compounds which are predominately C3, C4, and C5 aliphatic alcohols. n-Hexyl acetate is an additive in many foods because of

its fruity odor and is present in many fruits and alcoholic beverages and is known to be found in apples and plums. The last example compound Farnesene is an essential oil found on the coating of apples and is commonly used in flavoring food and drink.

Fig. 5 shows a comparison of two different apples with the first (A) being firm and crispy and the second (B) being soft and juicy. This can be deduced by the sharper, larger first "chew" in the mechanical characteristic curve for apple species A. Subsequent "chews" two through five also indicates apple species A to be more firm and crispy.

In addition to the textures being different the compounds released were also different. Table 2 shows the compounds released in apple species B as well as the percent increase in these compounds with "chewing". Apples species B contained additional compounds 1) Butyl acetate and 8) p-Allylanisol not found in apple species A. p-Allylanisol is a natural organic, phenylpropene compound found in herbs and used in preparation of fragrances. Also compounds 2) sec-Butylcarbinol and 3) Ethyl hexanoate found in apples species A were not found in apple species B. Thus the texture and flavor of these two apples will be quite different.

In this application note the apple texture and flavor were determined simultaneously by a "chewing" process. It was found that apples released mostly alcohols and esters. The flavor release evaluation kit can be used and an effective tool for food development. The texture/flavor test can be performed on many different types of foods to copy that homemade cooking from mom.
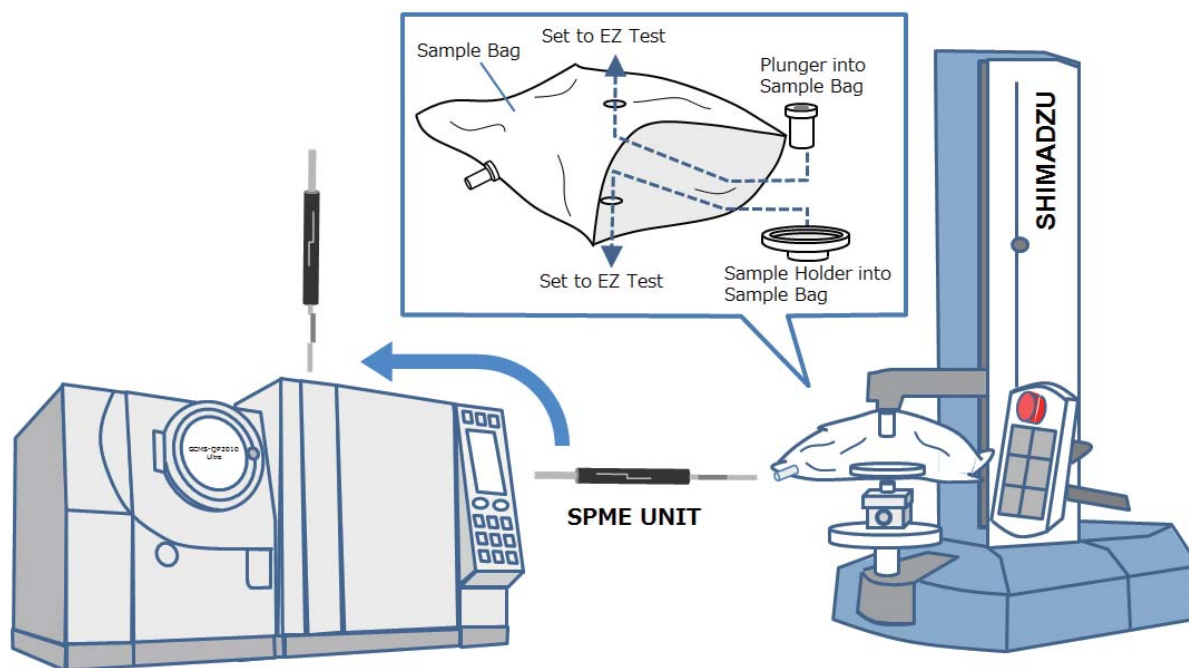

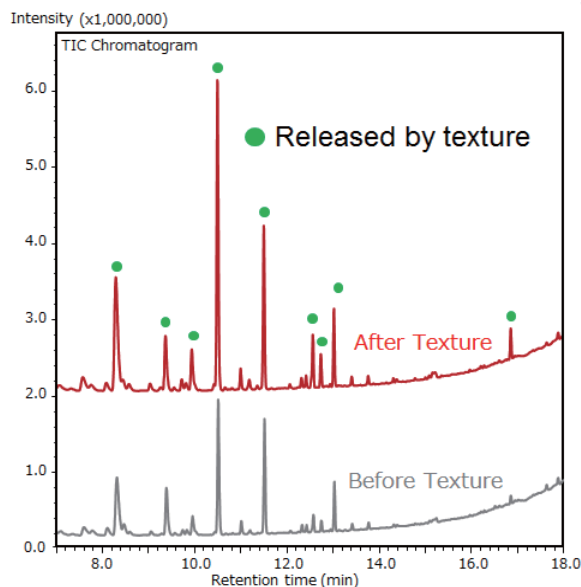Fig. 2 EZ Test Flavor Release Evaluation Kit

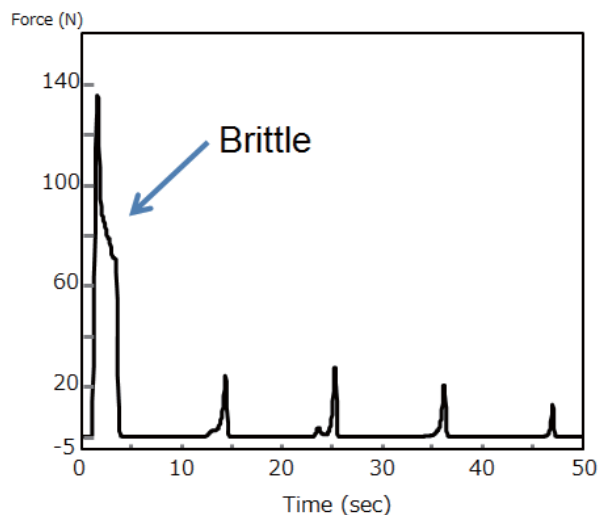Fig. 3 Results of Flavor Release Evaluation System on an Apple

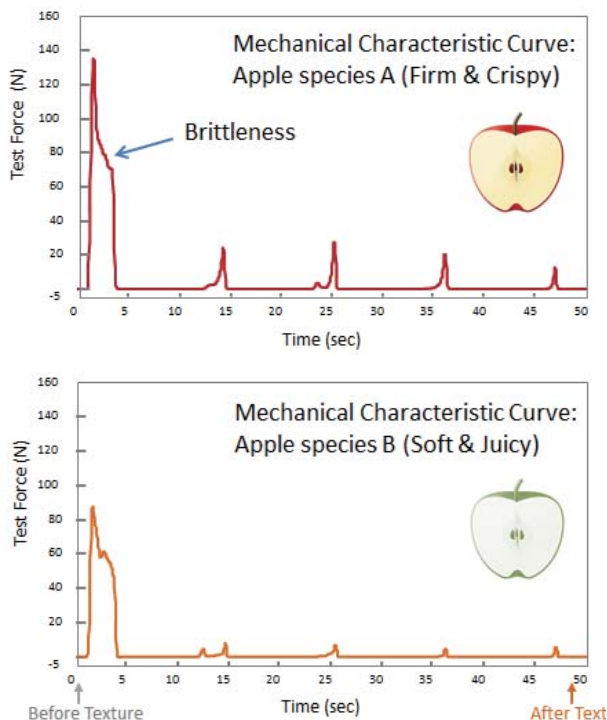Fig. 4 Mechanical Characteristics Curve of an Apple

Fig. 5 Mechanical Characteristic curves
for Apples Species A and B

Table 1 Increase in Compounds After
"Chewing" of Apple Species A

Peak No.	Compound name	Rate of increase (%)
1	2-Methylbutyl acetate	96
2	sec-Butylcarbinol	16
3	Ethyl hexanoate	108
4	n-Hexyl acetate	130
5	n-Hexanol	43
6	n-Hexyl butyrate	202
7	Hexyl 2-methylbutyrate	192
8	6-Methyl-5-hepten-2-ol	58
9	Farnesene	264

Table 2 Increase in Compounds After
"Chewing" of Apple Species B

Peak No.	Compound name	Rate of increase (%)
1	Butyl acetate	73
2	2-Methylbutyl acetate	99
3	n-Hexyl acetate	128
4	n-Hexanol	33
5	n-Hexyl butanoate	241
6	Hexyl 2-methylbutyrate	240
7	6-Methyl-5-hepten-2-ol	49
8	p-Allylanisole	139
9	Farnesene	192

Authors

Robert H. Clifford, Ph.D. is the Food and Consumer Products Business Unit Manager at Shimadzu Scientific Instruments. He can be reached at rhclifford@shimadzu.com

Satoshi Yamaki, Ph.D. is an Analytical Applications Development Engineer at Shimadzu Corporation and he can be reached at oreno@shimadzu.co.jp

Shimadzu Establishes Subsidiary in Malaysia

– Shimadzu Malaysia Sdn Bhd Will Strengthen Business Operations in the India Subcontinent and ASEAN Regions –

New Analytical and Measuring instruments manufacturing plant and in-house Application Laboratory are also scheduled to start operations soon

Shimadzu Corporation has established Shimadzu Malaysia Sdn Bhd, an indirectly-owned subsidiary, to further strengthen its capabilities in the India subcontinent and ASEAN region, which have shown remarkable economic growth in recent years and are projected to continue growing in the future.

This new company is a wholly-owned subsidiary of Shimadzu (Asia Pacific) Pte Ltd, abbreviated below as SAP (head office: Singapore, Managing Director: Kiminobu Imura). Shimadzu Corporation also plans to establish a new manufacturing facility for Analytical and Measuring instruments and an in-house Application Laboratory, in order to strengthen manufacturing, sales and application support capabilities. Construction on the new plant is scheduled to start in October 2015 and finish in July 2016, with operations starting in December 2016.

The ASEAN and India-subcontinent regions are key growth markets with sales of JP¥ 22 billion (US$ 187.18 million) estimated for FY 2016 in Shimadzu's medium-term management plan (FY 2014-to-FY 2016). Therefore, in order to leverage this massive growth potential, Shimadzu is building a solid business foundation by establishing a timely product supply system and is also developing extensive sales capabilities that can focus on the needs of the local markets.

Shimadzu Malaysia Sdn Bhd
– Where 140 Years of Global Knowledge Meets Vast Expertise of Local Distributors

Previously, analytical and measuring instrument sales in Malaysia were conducted through Shimadzu's local distributors. However, this newly established local subsidiary of Shimadzu will now provide direct sales and service capabilities. While starting direct sales of analytical instruments, such as High-Performance Liquid Chromatographs (HPLCs), to markets such as food products, pharmaceuticals, and petrochemical, Shimadzu will continue to rely on the expertise of local distributors for sales of X-ray Fluorescence spectrometers, optical emission spectrometers, X-ray diffractometers, testing machines, and non-destructive inspection systems. This will not only help in maximizing customer convenience but will also help to further build the brand image of Shimadzu.

Consequently, SAP's Malaysian branch office, which was established in 2000 for direct sales of medical equipment, will be absorbed and integrated into the new subsidiary as its medical systems department. Shimadzu has earned a strong reputation in Malaysia for the quality and extensive service of Shimadzu X-ray systems, which has resulted in the organization securing a 25% market share, with over 50% of the market for general-purpose X-ray systems sold to hospitals. Shimadzu now intends to strengthen efforts to sell more high-end Angiography systems and develop the clinic market.

Owing to these new capabilities, Analytical and Measuring instrument sales are projected to be US$ 15.05 million and Medical system sales US$ 5.20 million in 2018. In total, Shimadzu Malaysia Sdn Bhd is expected to garner sales of about US$ 20.25 million in 2018, which is double the sales recorded in 2013.

Overview of the Sales Subsidiary

1) Name: Shimadzu Malaysia Sdn. Bhd. (abbreviation: SML)
   Wholly owned by Shimadzu (Asia Pacific) Pte Ltd. (abbreviation: SAP)

2) Capital: 9.50 million ringgit (about JP¥ 304 million, at 1 ringgit = 32 ¥)

3) Head office location: Nouvelle Industrial Park 2, Petaling Jaya, Selangor, Malaysia

4) Facility size: About 945 m², of which 160 m² is an application laboratory

5) Managing Director: Koji Nishinaka

6) Number of employees: 44 in first year (1 representative, 19 analytical instrument personnel, 16 medical system personnel, and 8 administrators, including 1 Japanese expatriate)

7) Business description: Sales and service of analytical and measuring instruments and medical systems in Malaysia

8) Annual sales: About US$ 20.25 million planned for 2018

9) Start of operations: January 2, 2015

Malaysia Manufacturing Facility for Analytical and Measuring Instruments to Start Production in 2016

Shimadzu Corporation will establish a new analytical and measuring instrument manufacturing facility in Malaysia for SAP sales to South East Asia, the Indian subcontinent, and Oceania regions. This will improve SAP's ability to accommodate the particular needs of those markets, comply with regulations, and supply products in a more effective manner. Production of SAP's state-of-the-art products, which are expected to witness a substantial growth, such as the Prominence, Prominence-i, and Nexera High-Performance Liquid Chromatographs (HPLCs) and the UV-1800 UV-VIS spectrophotometer, is scheduled to start in 2016. Following these, production will be successively expanded to include other products and models. The facility will also supply some Liquid Chromatograph products to subsidiaries in charge of sales in the Middle East and Africa. This will provide product supply capabilities that are competitive in terms of quality, price, and delivery lead time, which will help expand sales in the medium and long term.

Shimadzu Nexera UC Recognized at Pittcon 2015 with Editors' Gold Award

From left to right: Terry Adams, VP Marketing, Shimadzu Scientific Instruments; Bob Clifford, Marketing Manager, Food, Shimadzu Scientific Instruments; Stefan Fritsch, editor Instrument News; Bob Stevenson, editor American Laboratory magazine. (Photo courtesy of Roy Engelbrecht, Engelbrecht Studios)

Shimadzu Corporation announced that its fully automated supercritical fluidic chromatography-based Nexera Unified Chromatography system (Nexera UC) received the Pittcon Editors' Gold award at the 2015 Pittsburg Conference on Analytical Chemistry and Applied Spectroscopy (Pittcon) held in New Orleans, LA between March 8th and 12th.

The Pittcon Editors' Awards recognize the most innovative products presented at Pittcon for the first time based on votes by the media corps. Editors awarded the Nexera UC for its ability to provide a workflow solution that addresses the bottleneck in real-world sample analyses.

The Nexera UC is the world's first fully automated system that combines on-line SFE and SFC in a single flow path. This unique system enables automatic extraction of up to 48 samples followed by seamless transfer to SFC/MS for high-sensitivity detection of targets by mass spectrometry.

The Nexera UC system eliminates the need for complicated sample preparation and enables highly reliable and stable analysis of delicate samples that are prone to oxidation or degradation if exposed to air and/or light. The fully automated Nexera UC system has a much higher target analyte recovery rate and reduces the possibility of human error during analysis when compared to conventional manual workflows and off-line systems. In addition, Nexera UC enables the analysis of compounds over a diverse chemical space and significantly reduces the quantity of organic solvents used.

"Shimadzu is extremely proud to receive the Pittcon editors' gold award, an achievement that serves to recognize all of the engineers and scientists who developed the Nexera UC," said Dr. Teruhisa Ueda, General Manager, Analytical & Measuring Instruments Division. "As the world's first fully automated system that combines on-line SFE and SFC in a single flow path, the Nexera UC will eliminate the need for complicated sample preparation and thereby improve productivity in the lab. We are eager to collaborate with customers around the world on this exciting new product."

For more information about the Nexera UC system, visit:
http://www.shimadzu.com/an/hplc/nexera_uc/index.html

Opening of the Osaka University and Shimadzu Analytical Innovation Research Laboratory Joint Laboratory Will Focus on Developing Cutting-Edge Technology for Comprehensively Investigating Biological Activities of Cells

Overview

Shimadzu Corporation and Osaka University have inaugurated the Osaka University and Shimadzu Analytical Innovation Research Laboratory (mentor professor: Eiichiro Fukusaki) at the Graduate School of Engineering, Osaka University, and have initiated full-scale research activities. The purpose of this laboratory is to establish and advance analytical techniques with respect to metabolomics. In particular, this laboratory will be engaged in research and development of data analysis platforms based on mass spectrometers. (From December 1, 2014 to March 31, 2017)

Purpose of Establishing the Research Laboratory

Metabolomics is a cutting-edge field of study for the comprehensive investigation of biological activities of cells, based on exhaustive detection and analysis of metabolic products in organisms, and the precise assessment of their behavior. In recent years, metabolomics has become a focus of attention due to its application to a variety of fields. For instance, in the field of medicine, application examples include the analysis of physiological and pathological mechanisms, and the search for disease biomarkers. In plant and food fields, examples include clarification of the stress response of plants and the functional analysis of foods. In the field of manufacturing, the improvement and optimization of fermentation productivity, as well as biofuel productivity improvements can be expected. At the same time, metabolomics involves a mixture of sciences and is a new research field that is still in its infancy in terms of developing technologies and operating methods. It requires further technological development.

Through many years of product development, Shimadzu has accumulated vast knowledge and expertise on mass spectrometry technology that demonstrates its merits in the simultaneous analysis of complicated and diverse components, and is applying this technology to advance responses to metabolomics-related needs. Professor Fukusaki at Osaka University is a leading researcher in the development and application of metabolomics, and has cutting-edge expertise with respect to pretreatment methods, analysis methods, and data analysis methods. By making use of the merits of the joint research course program system, where advanced cooperative research aimed at resolving issues shared by business and academia can

be performed, this research laboratory will address issues in metabolomics by integrating technology from both parties.
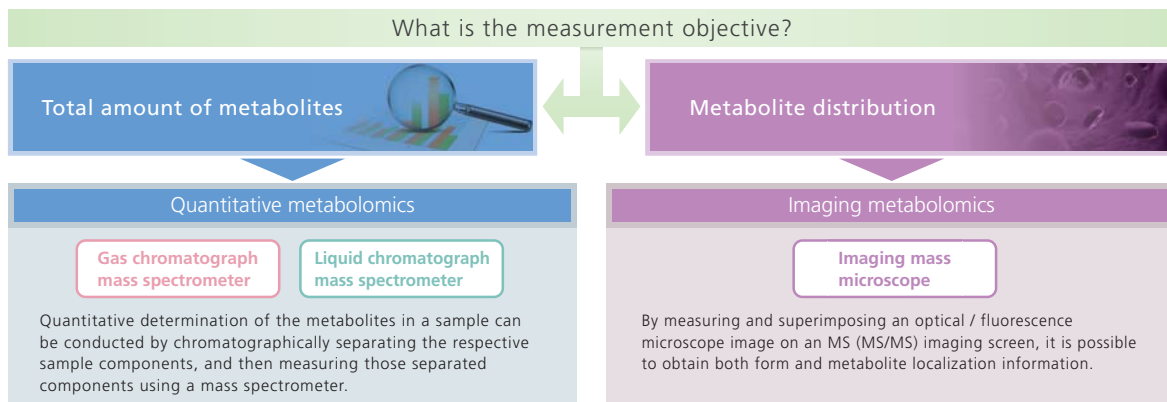
Subsequent Specific Measures

Using Shimadzu gas chromatograph-mass spectrometers, liquid chromatograph-mass spectrometers, supercritical fluid chromatograph-mass spectrometers, and imaging mass microscopes, the joint research activities of this research laboratory involve both quantitative metabolomics--measuring the total quantity of metabolites contained in samples, and imaging metabolomics--visualizing distribution information. The laboratory will investigate the optimal conditions for metabolomic techniques in a variety of fields and develop applications, with an aim of contributing to the further dissemination and advancement of metabolomics.

"Metabolomics, which involves a mixture of life sciences, organic chemistry, analytical chemistry, and information science, has only just started in terms of developing technologies and operating methods," said Professor Eiichiro Fukusaki, Graduate School of Engineering, Osaka University. "Therefore, we are developing new technologies for metabolomic data analysis systems and researching new operating methods. Through this Analytical Innovation Research Laboratory, an industry-academic cooperative initiative promoted by Osaka University, we are aiming to contribute to society, not only in Japan, but also in countries in Southeast Asia and other areas."

"Metabolomics has attracted considerable interest as a promising means of understanding fundamental biology and elucidating the function of genes with unknown functions. Furthermore, it may also be useful for medical treatments, foods, and the molecular breeding of industrial microorganisms," said Teruhisa Ueda, General Manager, Analytical & Measuring Instruments Division, Shimadzu Corporation. "It will be important to develop the optimal methods and applications to suit these objectives. Through this joint research laboratory with Osaka University, we will strive for new problem-solving techniques and aim for revolutionary breakthroughs, while redoubling efforts to create returns to society, including human resources."

For more details, visit:
http://www.shimadzu.com/labcamp/

What is the measurement objective?

Total amount of metabolites	Metabolite distribution
Quantitative metabolomics	Imaging metabolomics
Gas chromatograph mass spectrometer / **Liquid chromatograph mass spectrometer**	**Imaging mass microscope**
Quantitative determination of the metabolites in a sample can be conducted by chromatographically separating the respective sample components, and then measuring those separated components using a mass spectrometer.	By measuring and superimposing an optical / fluorescence microscope image on an MS (MS/MS) imaging screen, it is possible to obtain both form and metabolite localization information.

Mass Spectrometers Used in Metabolomics

RF-6000

Shimadzu's New RF-6000 Spectrofluorophotometer Combines Superior Analysis Capabilities with Enhanced Ease of Use

Click here>>

By combining new technologies with those cultivated over Shimadzu's long history, the Shimadzu spectrofluorophotometer has been reborn as the RF-6000. Combined with new LabSolutions RF software, designed for unrivalled measurement accuracy and easy operation, the RF-6000 offers the ultimate performance for a diverse range of customers' measurement needs.

Features
- Wide Variety of Spectral Techniques
- High Sensitivity, High Stability and High Speed
- Excellent Usability

ECD-2010 Exceed

The World's Highest Level of Durability and Performance for a Capillary ECD

Click here>>

The newly designed capillary ECD cell incorporates Shimadzu's proprietary Contact Free Technology. This technology significantly reduces deposition of sample residue on the detector and radiation source. The new ECD has been optimized at all levels, enabling truly world-class performance. The ECD-2010 Exceed provides solutions for PCB analysis, analysis of halogenated contaminants in water and wastewater, and agrochemical residue analysis.

Features
- Long Operating Life
- High-Performance Specifi cations

UV-1280

Monitored, Single-Beam UV-Vis Spectrophotometer Offers Comprehensive Measurement Options in a Compact Body

Click here>>

Offering wavelength scanning from 190 to 1,100 nm, this compact, high-quality instrument is ideal for applications in a variety of industries, including environmental, food quality, and life science.

Features
- Easy to Operate
- A Variety of High-Level Measurement Modes
- Data Storage on USB Flash Drives

PPSQ-31B/33B

Achieving Greater Simplicity and Reliability in the Determination of Amino Acid Sequences

Click here>>

The PPSQ-31B/33B continues the tradition of providing reliable and sensitive N-terminal protein sequencing to researchers through automated Edman Degradation.

Features
- Analysis of PTH-Amino Acids Using Isocratic Mode
- Simple Operations

Looking for clever minds!
'Lab4You' program for young scientists

With the 'Lab4you' program, Shimadzu offers young scientists from all over Europe the opportunity to win laboratory bench space in the ultra-modern 'Shimadzu Laboratory World' in Duisburg, Germany. There, they will have access to the latest analytical HPLC/UHPLC and mass spectrometry instruments to achieve the best analytical results for their research. They will be supported by product specialists from the Shimadzu Europa GmbH. Preconditions for participation are an undergraduate degree in the domain of science, an interesting research topic and previous knowledge of HPLC/UHPLC and/or mass spectrometry. Master students, PhD students and post-docs from all scientific disciplines where chromatography and mass spectrometry are used can apply. The winner is selected by an internal jury and will subsequently be notified.

Applications in English language may be submitted via www.shimadzu.eu/lab4you with a short abstract of the research project.

Shimadzu welcomes your idea and input!
►► Questionnaire

Find us on
Facebook

SHIMADZU

Shimadzu Corporation
www.shimadzu.com/an/