SHIMADZU
Excellence in Science

Analysis Solutions to Fight Food Fraud

SHIMADZU
Excellence in Science

Application News

No. B84

MALDI-TOF Mass Spectrometry

Simple and Rapid Identification of Vegetable Oils Using a Benchtop MALDI-TOF Mass Spectrometer and eMSTAT Solution™ Statistical Analysis Software

Matrix-assisted laser desorption/ionization time-of-flight mass spectrometers (MALDI-TOF MS) feature simple and rapid detection of low molecular to high molecular weight compounds in a wide range of samples. MALDI-TOF MS is widely used to determine molecular weights of synthesized products and natural substances in R&D laboratories and quality control sectors. In addition, utilizing the feature of MALDI-TOF MS which can detect multiple components in a wide mass range as singly-charged ions (1 component = 1 peak), an attempt is being made to use the instrument for profiling property changes of food and biological specimens.

This article introduces an example of simple and rapid identification of vegetable oils, using a benchtop MALDI-TOF MS and the eMSTAT Solution statistical analysis software.

K. Shima

Materials and Methods

Samples were prepared by diluting six kinds of commercially available vegetable oils (three olive oils, flaxseed oil, sunflower seed oil, and grape seed oil) to 1 mg/mL using chloroform. Each sample was mixed with an equal volume of matrix solution and cationization agent, spotted onto a reusable MALDI target slide and dried.

The matrix was prepared by dissolving 2,5-dihydroxybenzoic acid (DHB, 10 mg/mL) in methanol, and the cationization agent was prepared by dissolving sodium iodide (1 mg/mL) in tetrahydrofuran. Samples were analyzed using the MALDI-8020 benchtop MALDI-TOF mass spectrometer (Fig. 1). The peak lists obtained from the mass spectra were subjected to multivariate analysis using the eMSTAT Solution software to identify each oil type.

Results

The mass spectra of six vegetable oil samples are shown in Fig. 2. Mainly sodium-adducted molecules originated from diacylglycerols (DAGs) are detected near *m/z* 600, and triacylglycerols (TAGs) are detected near *m/z* 900.

The major constituent fatty acids of the detected TAGs are inferred based on references as listed in Table 1.

Fig. 1 MALDI-8020 Benchtop MALDI-TOF MS

Table 1 Compounds Inferred from Major Peaks Detected in the Mass Spectra of Vegetable Oils[1), 2)]

Observation *m/z*	Inferred Compounds
873.7	C55:6 (PLnLn)*
875.7	C55:5 (PLLn)
877.8	C55:4 (PLL/PLnO)
879.8	C55:3 (PLO/PLnS)
881.5	C55:2 (POO/PLS)
895.6	C57:9 (LnLnLn)
897.7	C57:8 (LLnLn)
899.7	C57:7 (LLLn)
901.5	C57:6 (OLLn/SLnLn)
903.8	C57:5 (OOLn/OLL)
905.5	C57:4 (SLL/OOL)
907.5	C57:3 (OOO/SOL)

* (In parentheses) L = linoleic acid, Ln = linolenic acid, O = oleic acid, P = palmitic acid, S = stearic acid

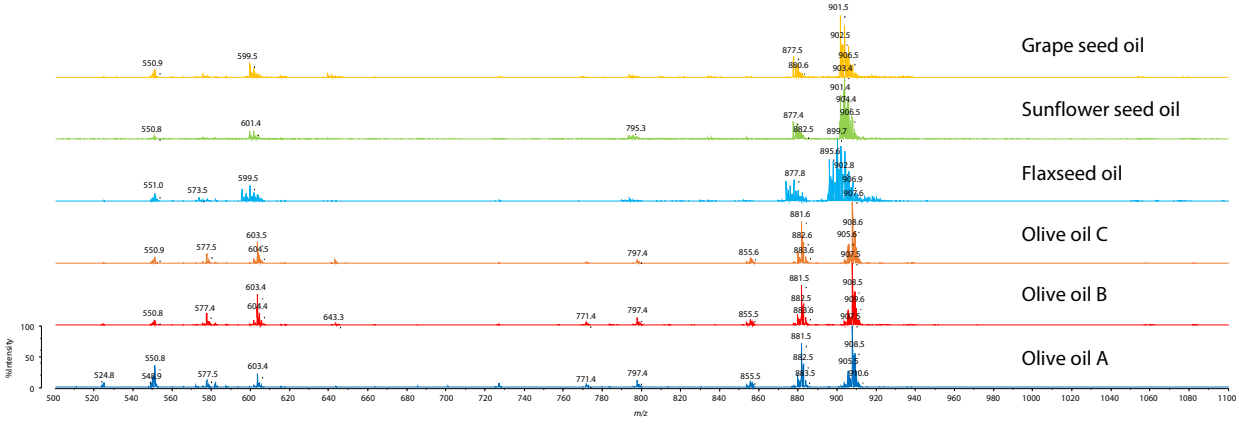


Fig. 2 Mass Spectra of Vegetable Oils

Fig. 3 shows the results (score plot) of multivariate analysis (algorithm: PLS-DA) of the peak lists of vegetable oil samples, which were detected by the MALDI-TOF mass spectrometer, using the eMSTAT Solution software. The samples can be classified into three groups: three olive oils of the high oleic type, flaxseed oil of the high linolenic type, sunflower seed oil and grape seed oil of the high linoleic type, indicating the major constituent fatty acid of each vegetable oil. From the results of the loading plot (Fig. 4), we can see that peaks at *m/z* 907 of the high oleic type, those at *m/z* 903 of the high linoleic type, and those at *m/z* 899 of the high linolenic type contribute to the grouping.

Next, we created a discrimination model (algorithm: Random Forest) to enable discrimination of the acid type of the major constituent fatty acid in an unknown vegetable oil using the measured data of the vegetable oil samples (upper figure in Fig. 5). We used the discrimination model to perform discriminant analysis of olive oils made by different manufacturers (other than those of the oils used for creating the model). All samples are identified correctly as the high oleic type (lower figure in Fig. 5).

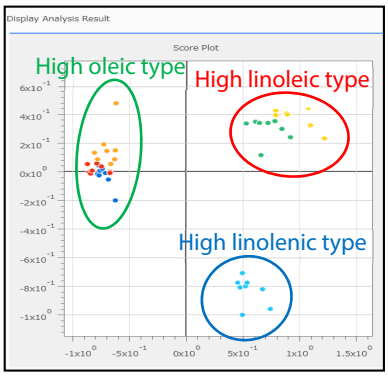


Fig. 3 Results of Multivariate Analysis of Vegetable Oils (Score Plot)
●Olive oil A ●Olive oil B ●Olive oil C ●Flaxseed oil ●Sunflower seed oil ●Grape seed oil

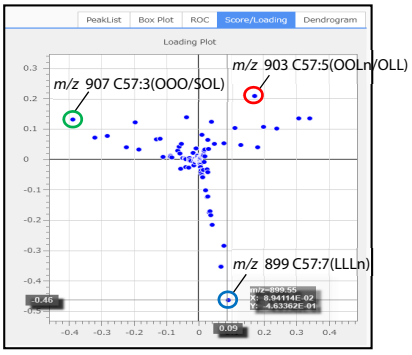
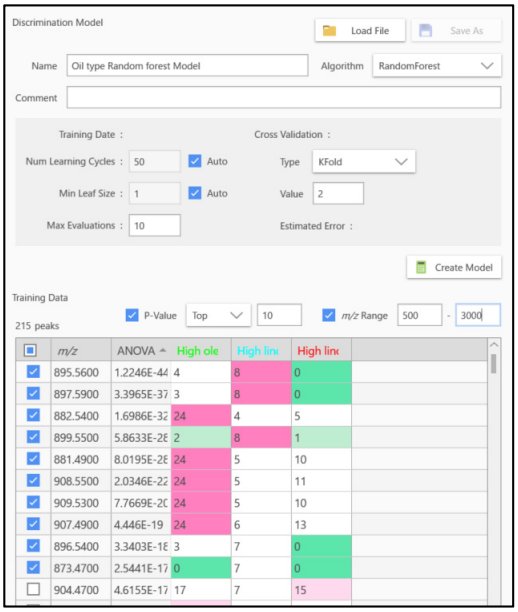
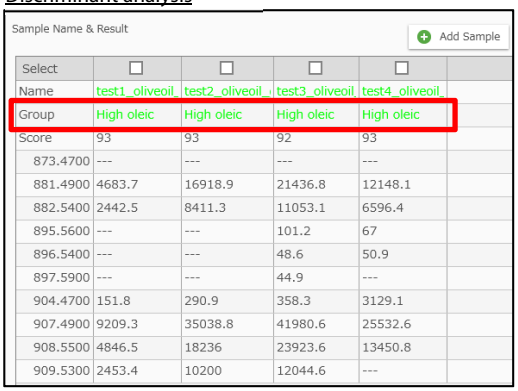


Fig. 4 Results of Multivariate Analysis of Vegetable Oils (Loading Plot)

Creation of discrimination model



Discriminant analysis



Select				
Name	test1_oliveoil_	test2_oliveoil_	test3_oliveoil	test4_oliveoil_
Group	High oleic	High oleic	High oleic	High oleic
Score	93	93	92	93
873.4700	---	---	---	---
881.4900	4683.7	16918.9	21436.8	12148.1
882.5400	2442.5	8411.3	11053.1	6596.4
895.5600	---	---	101.2	67
896.5400	---	---	48.6	50.9
897.5900	---	---	44.9	---
904.4700	151.8	290.9	358.3	3129.1
907.4900	9209.3	35038.8	41980.6	25532.6
908.5500	4846.5	18236	23923.6	13450.8
909.5300	2453.4	10200	12044.6	---

Fig. 5 Discriminant Analysis of Vegetable Oils (Upper: created discrimination model, Lower: discriminant analysis results of olive oils)

Conclusion

This article demonstrates that simple and rapid grouping of vegetable oils by the major constituent fatty acid can be performed using the MALDI-8020 benchtop MALDI-TOF mass spectrometer and statistical analysis software. In this analysis, fats were measured and we assume that this technique can be applied to a variety of samples including proteins, glycans, synthetic products, and biological specimens.

The benchtop MALDI-8020 features compactness and capabilities sufficient for molecular profiling, and its future dissemination in simple and rapid evaluation of properties in versatile samples is expected.

<References>
1) Ohtani, H, Yamahashi, Y, Ishida, Y: 2010. Curing Behavior Analysis of Drying Oil using Matrix-assisted Laser Desorption Ionization Mass Spectrometry (in Japanese). 58th Annual Conference on Mass Spectrometry, Japan
2) Picariello G, Paduano A, Sacchi R, Addeo F, 2009. Maldi-tof mass spectrometry profiling of polar and nonpolar fractions in heated vegetable oils. *J. Agric. Food Chem.*, 57 (12), pp. 5391-5400

First Edition: Oct. 2018

Shimadzu Corporation
www.shimadzu.com/an/

SHIMADZU
Excellence in Science

Application News

No. AD-0167

Halal Authentication Analysis / IRTracer-100

Quantitative Determination of Lard Adulteration by FTIR Spectroscopy with Chemometrics Method - Virgin Coconut Oil

Zhen Hao Lee, Jia Sheng Kuek*, Joyce Lim, Ai Ming Chua
Application Development & Support Centre, Shimadzu (Asia Pacific) Pte Ltd, Singapore
**ITS Student from Nanyang Technological University, Singapore*

❑ Introduction

In recent years there has been a considerable amount of interest in virgin coconut oil (VCO) due to its potential health benefits [1]. Moreover, VCO has a higher commercial value compared to other edible oils. This has led to VCO to be a target for potential adulteration with cheaper oil or animal fat such as lard. Lard resembles VCO due to its creamy white and solid form appearance at room temperature. Such adulteration practice is a commercial fraud which could have a negative economic implication. Therefore, there is a need to establish a reliable method to detect and quantitate lard adulteration in VCO.

Fourier Transform Infrared (FTIR) spectroscopy is a useful technique in identification of organic component due to its characteristic absorption in the infrared (IR) region. Furthermore, when being used in conjunction with chemometrics data analysis such as partial least squares (PLS) regression, it enables a rapid quantitative analysis with minimal sample preparation. This application news examines two measurement and quantitation methods, attenuated total reflection (ATR) with PLS quantitation and transmission with calibration curve quantitation, for the determination of lard adulterated VCO using FTIR spectroscopy.

❑ Experimental

Pork lard and commercially available VCO were purchased from local markets. The lard was extracted based on the procedure by Rohman and Che Man [2]. A set of 17 standards containing 0.5 – 30% (w/w) lard in VCO was prepared. This set of standards was measured using two different methods – ATR and transmission method.

In FTIR-ATR analysis, the sample was measured with horizontal type ATR attachment with zinc selenide (ZnSe) prism. Each sample was measured three times. The IR spectra were acquired in the wavenumber range of 4000 cm^{-1} – 650 cm^{-1}. The measurement conditions are shown in Table 1.

A PLS calibration model for lard was established with 15 of these standards using LabSolutions IR workstation with Chemometrics PLS function. The remaining 2 standards were used as samples for quantitative determination.

Table 1: Instruments and analytical conditions

Instruments	: IRTracer-100, ATR-8200H (ZnSe)
Resolution	: 4.0 cm^{-1}
Accumulation	: 45
Apodization	: Happ-Genzel
Detector	: DLATGS

In FTIR transmission analysis, Specac Pearl™ liquid transmission accessory, as shown in Figure 1, with a path length of 100 µm was used. A drop of the sample was added to the bottom ZnSe window, and the droplet was then sandwiched with the top window. This process is easier and faster than using traditional liquid fixed thickness cell. Each standard was measured once and the IR spectra was measured ranging from 4000 cm^{-1} – 650 cm^{-1}. The measurement conditions are shown in Table 2.

Table 2: Instruments and analytical conditions

Instruments	: IRTracer-100, Pearl™ (ZnSe, 100 µm)
Resolution	: 4.0 cm^{-1}
Accumulation	: 45
Apodization	: Happ-Genzel
Detector	: DLATGS

Figure 1: Specac Pearl™ liquid transmission accessory

❑ Results and Discussion

FTIR-ATR with PLS Quantitation Method

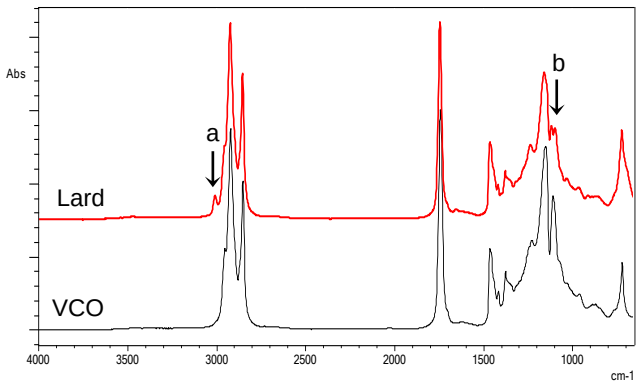


Figure 2: IR spectra of VCO and lard

Figure 2 shows the IR spectra of VCO and lard. The IR spectra of VCO and lard were quite similar, however there are some differences in the IR regions as indicated by arrows in Figure 2. A peak is present at around 3007 cm^{-1} for lard as shown by arrow (a) and is not observed for VCO. Peak at 3007 cm^{-1} is attributed to *cis* –C=H vibration and correlated with the presence of unsaturated fatty acid [3]. Coconut oil contains more than 90% of saturated fatty acid. Hence, a presence of peak at 3007 cm^{-1} may indicate an adulteration in coconut oil. In addition, at around IR range of 1120 – 1090 cm^{-1}, lard has two peaks at around 1097cm^{-1} and 1117 cm^{-1}, whereas VCO has only one peak at around 1110 cm^{-1} as shown by arrow (b). Figure 3 shows the IR spectra of VCO and lard in the IR range of 1220 – 1050 cm^{-1}.

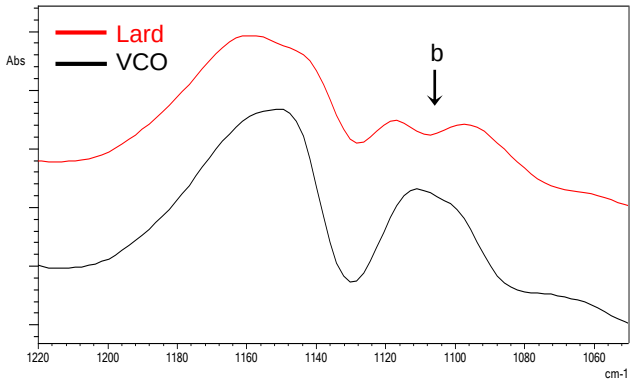


Figure 3: IR spectra of VCO and lard in IR range 1220 – 1050 cm^{-1}

A PLS calibration model was created using calibration standards of lard in VCO with concentration ranging from 0.5 – 30% w/w. Second derivative spectra were used in the PLS data analysis for better resolution of overlapping and shoulder peaks. Table 3 and Figure 4 show the PLS calibration parameter and result of lard in VCO.

Table 3: PLS calibration parameters of lard in VCO

Calibration Table	
Algorithm	PLS I
Number of references	51 (three measurement per sample)
Range (cm^{-1})	900 – 1500 2750 – 3050
Pre-process	Derivative, Order = 2, Points = 5
Scale	Autoscale
Number of factors	4
Square of correlation coefficient	0.9999
MSEP	0.0001
SEP	0.0098

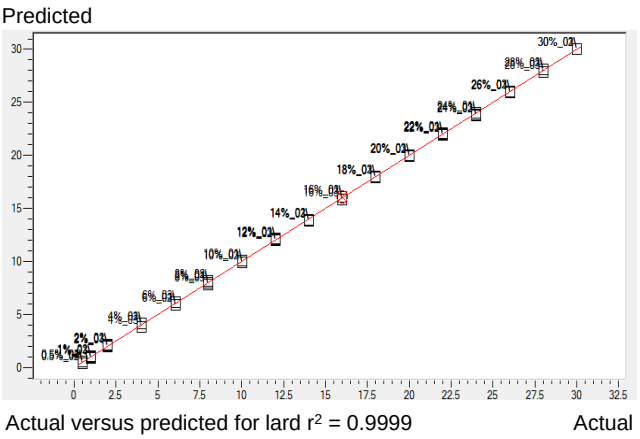


Figure 4: PLS calibration for lard in VCO predicted versus actual values

A good square of correlation coefficients of more than 0.999 was obtained for the PLS calibration modelling with low Mean Squared Error of Prediction (MSEP) and low Standard Error of Prediction (SEP).

Table 4 shows the quantitation results of lard in two different brands of VCO by PLS quantitation method. The measurement results are within ±10% of the expected values for lard in VCO.

Table 4: Predicted values of lard in two brands of VCO

Edible Oil		**Brand A VCO**		**Brand B VCO**	
Expected Value (% w/w)		2.5	5.0	2.5	5.0
Predicted Value (% w/w)	**1**	2.36	4.93	2.53	5.33
	2	2.44	5.31	2.62	5.29
	3	2.55	5.37	2.62	5.23
	Mean	2.45	5.20	2.59	5.28
Recovery (%)		98.0	104.1	103.6	105.7

Transmission with Calibration Curve Quantitation Method

A calibration curve was generated using 6 calibration standards of lard in VCO with concentration ranging from 1 – 10% w/w. The peak at around 3007 cm^{-1} from lard is selected for calibration. Figure 5 shows the IR spectra of lard in VCO calibration standards in the IR range of 3030 – 2992 cm^{-1}.

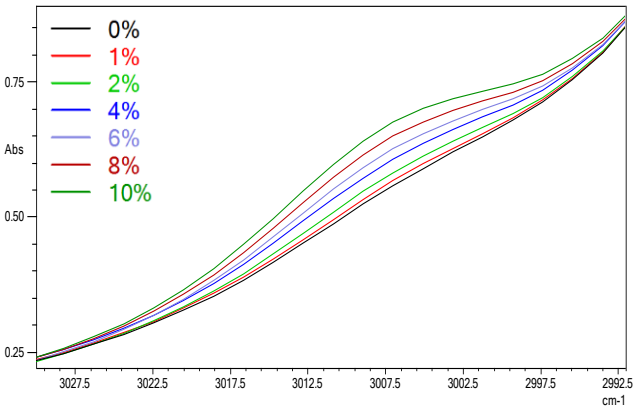


Figure 5: IR spectra of lard in VCO calibration standards in IR range 3030 – 2992 cm^{-1}

Table 5 shows the parameter setting for the generation of the calibration curve. A good square correlation coefficient of 0.997 was obtained. The calibration curve is shown in Figure 6.

Table 5: Calibration curve parameters of lard in VCO

Calibration Curve Parameters	
Component	Lard in VCO
Unit	%
Quantitation Method	Peak Area
Range (cm^{-1})	2992 – 3030, BC: ON
Calibration Curve	Multi-point
Order	1st order
Square of correlation coefficient	0.9973

Table 6 shows the quantitation results of lard in two different brands of VCO based on the transmission method. The measurement results are within ±10% of the expected values for lard in VCO.

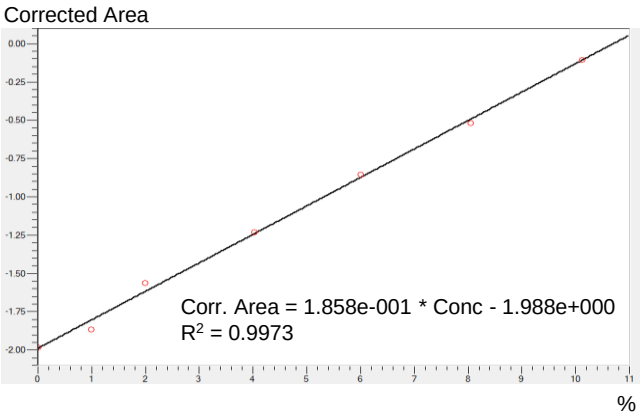


Figure 6: Calibration curve of lard in VCO

Table 6: Quantitation results of lard in two brands of VCO

Edible Oil		Brand A VCO		Brand B VCO	
Expected Value (% w/w)		2.5	5.0	2.5	5.0
Predicted Value (% w/w)	**1**	2.42	5.15	2.33	5.22
	2	2.52	5.20	2.31	5.24
	3	2.66	5.37	2.27	5.20
	Mean	2.53	5.24	2.30	5.22
Recovery (%)		101.3	104.8	92.1	104.4

❑ Conclusions

Using either FTIR-ATR with PLS quantitation method or transmission with calibration curve quantitation method, a good square correlation coefficient of more than 0.995 was obtained with a good recovery of within ±10% for the verification samples. This shows both methods have potential for quantitative determination of lard adulteration in VCO.

❑ References

1. Marina, A.M., Che Man, Y.B. and Amin, I. (2009). Virgin coconut oil: emerging functional food oil. Trends in Food Science and Technology, 20: 481-487.
2. Rohman, A. and Che Man, Y. B. (2009). Analysis of cod-liver oil adulteration using Fourier transform infrared (FTIR) spectroscopy. Journal of the American Oil Chemists' Society, 86, 1149–1153.
3. Rohman, A. and Che Man, Y. B. (2011). Simultaneous quantitative analysis of two functional food oils, extra virgin olive oil and virgin coconut oil using FTIR spectroscopy and multivariate calibration. International Food Research Journal 18(4): 1231-1235.

SHIMADZU
Excellence in Science

Application Development & Support Centre, SHIMADZU (Asia Pacific) Pte. Ltd, 79 Science Park Drive, #02-01/08 Cintech IV, Singapore 118264, www.shimadzu.com.sg; Tel: +65-6778 6280 Fax: +65-6778 2050

SHIMADZU
Excellence in Science

Application News

No. B106

MCE

Identification of Meat Species in Food Products by Molecular Biological Methods

Based on the Food Sanitation Act, Japan Agricultural Standard Law (JAS Law) and other related laws, producers and distributors of meat and meat products are required to display the place of origin, source species of meat, and part of the animal in order to protect the security and safety of foods, while the Islamic and Jewish religions strictly forbid consumption of pork for religious reasons. Since information concerning meat species contained in fresh meat and processed meat products is extremely important, a technology for identifying meat species is needed in order to assure product quality and the peace-of-mind of various consumers.

Methods for meat species indentification include protein-based methods (e.g., ELISA: Enzyme Linked Immunosorbent Assay) and molecular biological methods (PCR: Polymerase Chain Reaction). Protein-based methods are comparatively simple and analysis is inexpensive, but they are not suitable for identification of closely-related species or for analysis of processed food products. On the other hand, analysis of processed foods is considered possible by molecular biological methods because DNA has relatively high thermal stability. In meat species identification from their genetic characteristics, the cytochrome b gene region of mitochondrial DNA (mtDNA) is used as the target sequence. This article introduces an example of DNA detection in meat from beef, pork, chicken, lamb, horse meat, goat meat, and an analysis example in which the meat species were identified from processed meat products.

Y. Sogabe

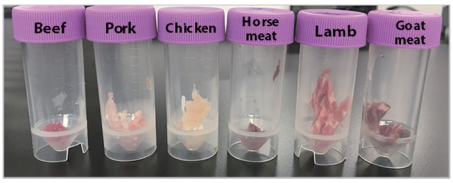


Fig. 1 Meat Samples: Beef, Pork, Chicken, Horse Meat, Lamb, Goat Meat

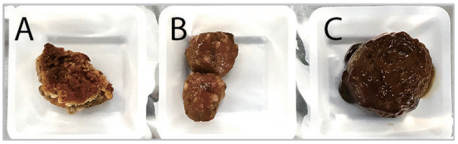


Fig. 2 Prosessed Meat Products (A, B, C)

Samples and Pretreatment

The samples used here were beef, pork, chicken, lamb, horse meat, goat meat and three kinds of processed meat products (A, B, C). The processes from sample pretreatment to identification of the meat species were carried out according to the protocol in Fig. 3.

First, 100 μL of the lysis buffer (Table 1) was added to a 5 mg single meat sample. For the processed meat samples, 500 μL of the solution was added to 50 to 100 mg of the sample. Zirconia beads with a size of φ2 mm were added to the above-mentioned sample solution, and the specimen was disintegrated under conditions of 5,000 rpm, 30 s, and 25 °C using a bead-type cell disruption system. The sample solution was then centrifuged at 5,000 rpm for 5 min at 25 °C and solids were removed as far as possible.

The supernatant was transferred to a different tube, and the proteinase K was inactivated by heating at 95 °C for 5 min. This sample solution was used as the PCR template sample.

Table 1 Lysis Buffer

Tris·HCl pH8.0	20 mM
EDTA	5 mM
NaCl	400 mM
SDS	0.30%
Proteinase K	200 μg/mL

PCR

A 0.5 μL of the sample solution obtained by pretreatment was used as the PCR template. The PCR method referred to the paper by Matsunaga et al. (Journal of the Japanese Society for Food Science and Technology, 46(3), 187, 1999). The composition of the PCR reaction mixture and the PCR program were as shown in Table 2.

Table 2 PCR Conditions

Reaction mixture		PCR program	
2x Ampdirect™ plus	10 μL	95 °C, 10 min	
BIOTAQ™	0.5 U	94 °C, 30 sec	35 cycles
primer-F	2 μM	60 °C, 60 sec	
primer-R	2 μM	72 °C, 90 sec	
Distilled Water	up to 20 μL	72 °C, 7 min	

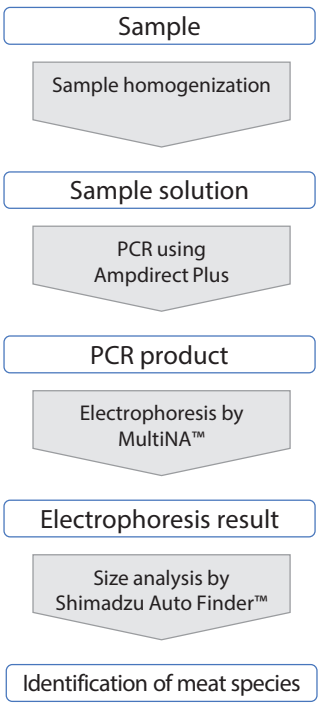


Fig. 3 Analysis Procedure

Electrophoresis and Identification of Meat Species

Electrophoresis of the PCR product was conducted with a MCE-202 MultiNA microchip electrophoresis system, and the size was confirmed. A MultiNA-dedicated DNA-500 Kit was used in the analysis with the MultiNA. For the analysis of the processed meat product samples, a Shimadzu Auto Finder was used to detect the sizes specific to the meat species, and the meat species were identified.

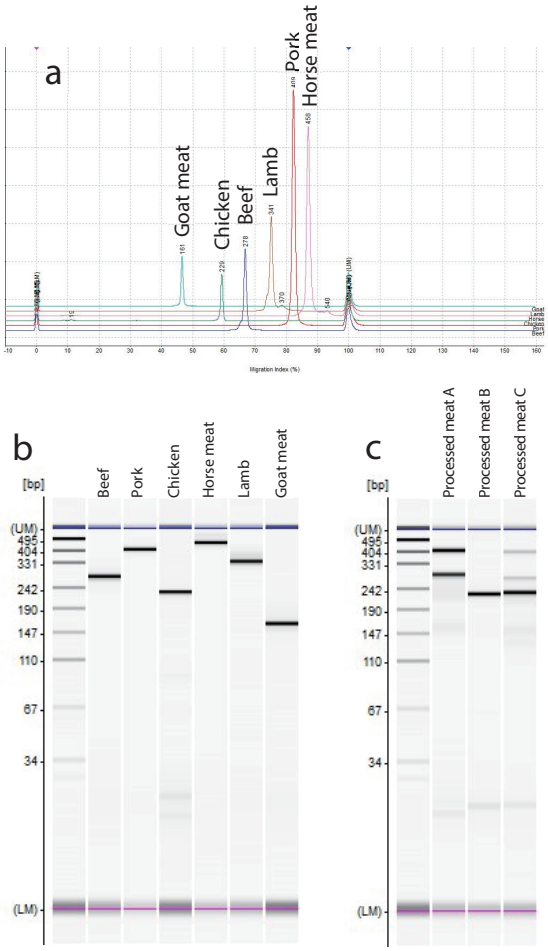
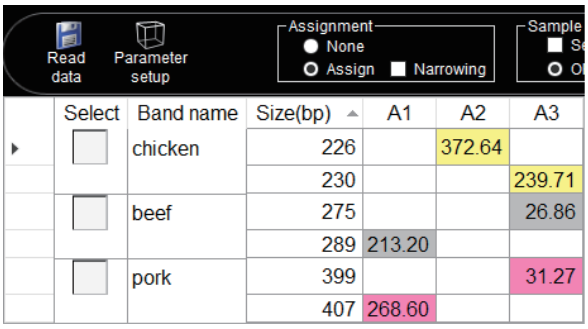


Fig. 4 Electrophoresis of PCR Products by MultiNA (Electropherogram and Gel Image)
a: Electropherogram of Meat Samples
b: Gel Image of Meat Samples
c: Gel Image of 3 Processed Meat Products



Select	Band name	Size(bp)	A1	A2	A3
	chicken	226		372.64	
		230			239.71
	beef	275			26.86
		289	213.20		
	pork	399			31.27
		407	268.60		

Fig. 5 Result of Identification of Meat Species of Prosessed Meats by Shimadzu Auto Finder
A1: Processed meat A, A2: Processed meat B, A3: Processed meat C
Numerical values in data indicate peak intensity (mV).

Results

Fig. 4 shows the results of the analysis of the six kinds of meats and the three kinds of processed meat products. With the method proposed by Matsunaga et al., the sizes of the DNA amplified by PCR from beef, pork, chicken, lamb, horse meat and goat meat are considered to be 274 bp, 398 bp, 227 bp, 439 bp, 331 bp, and 157 bp, respectively. These could also be detected clearly in this analysis (Fig. 4a, b).

In the processed meat products, two DNA fragments were detected from A, one was detected from B, and three were detected from C (Fig. 4c). When the data obtained by the MultiNA were analyzed with the Shimadzu Auto Finder, beef and pork were identified from A, chicken was identified from B, and chicken, beef, and pork were identified from C (Fig. 5). Samples B and C had commercial packages, and the contents identified in the analysis were the same as the source meat species indicated on the packages.

Conclusion

Extraction and purification of DNA is normally necessary in a molecular biological method. However, these processes are complex, and are time-consuming when a large number of samples is required. On the other hand, Ampdirect Plus has a neutralizing action for the PCR inhibitors protein and sugar in samples and enables direct PCR from the sample without DNA purification.

In electrophoresis by the MCE-202 MultiNA, a fully-automatic analysis was possible simply by setting the reagents and samples.

The Shimadzu Auto Finder, which is optional software for the MCE-202 MultiNA, can detect DNA of designated sizes from the digital data outputted by the MultiNA.

In conclusion, simple molecular biological identification of meat species is possible by using a combination of Ampdirect and the MultiNA system.

First Edition: Nov. 2019

SHIMADZU
Shimadzu Corporation
www.shimadzu.com/an/

Qualitative and quantitative analysis of pork in beef food with LC-MS/MS

Introduction

Food fraud is a problem on global scale, especially the meat fraud. Such as, horse meat was added in the beef food, and pork was used as beef. Consumers and the food industry are increasingly aware of this problem, and the specific detection of amounts of certain meat species in processed food is still problematic. Presently, ELISA and PCR methods are routinely used for the species authentication. As we know, The ELISA method often results in false positive or false negative results, and the PCR method is not so accuracy for DNA being prone to degradation. So the characteristic peptides multiple reaction monitoring (MRM) method by LC-MS/MS is a good choice for the qualitative and quantitative analysis of meat authentication.

Methods and Materials

Sample preparation:

The meat was cut into slices and minced using an electric meat grinder. Approximately 2 g of sample material (beef spiked with pork at the ratio of 5%, 10%, 20%, 40%, 60% and 80%) was weighed in a centrifuge tube (50 mL), and 10 mL of extraction buffer (7 M urea, 2 M thiourea, and 50 mM Tris-HCl, pH 8) was added. All samples were vortexed for 20 s and extracted using an Ultra Turrax T-25 (IKA, Germany) with a 10 N dispersing element. Samples were dispersed for 30 s at 8000 rpm, followed by 30 s at 9000 rpm and finally 30 s at 11000 rpm. Following extraction, samples were centrifuged for 30 min at 4 °C at 20000 rpm. After extraction, the samples were reacted with DTT and IAA, and then digested with trypsin. After digestion, the samples were pretreated with HLB SPE column to obtain the samples for analysis.

Analysis conditions

Instruments	: LC-30A+LCMS-8050
LC condition	
Column	: Shim-pack GISS (2.0 mm I.D.×150 mm L., 2.1 µm) ;
Mobile phase	: A-water+0.1% formic acid ; B-ACN+0.1% formic acid ;
Binary gradient	: 5%B (0 min)-50%B (12 min)-80%B (12.01-14 min)- 5%B (14.5 min)-Stop (18 min);
Flow rate	: 0.3 mL/min;
Column temperature	: 40 °C;
Injection volume	: 5.0 µL
MS condition	
Ion type	: ESI+;
Scan mode	: MRM;
Interface temp.	: 300 °C
DL temp.	: 250 °C
Heating block temp.	: 400 °C
Nebulizing gas	: 3 L/min
Drying gas	: 10 L/min
Heating gas	: 10 L/min
Detector voltage	: Tuning result
Dwell time	: 10-20 ms

Qualitative and quantitative analysis of pork in beef food with LC-MS/MS

Figure 1. Shimadzu LCMS-8050

Result

CE energy optimizing for characteristic peptides

According to the related report, these four peptides SALAHAVQSSR, TLAFLFAER, YDIINLR, LVVITAGAR were selected as the characteristic peptides for port, and TLALLFSGPASGEAEGGPK, EASGPINFTVFLNMFGEK, HPSDFGADAQAAMSK, ALEDQLSELK, LVIITAGARF were selected as the characteristic peptides for beef. In order to establish good quantitative method, the CE energy was optimized with the software Skyline (Figure 2 and 3). In consideration of the optimizing results and the matrix effect the characteristic peptides TLAFLFAER (m/z 534.30>853.45) and TLALLFSGPASGEAEGGPK (m/z 901.45>1290.60) were selected as the quantitative peptides for pork and beef, respectively.

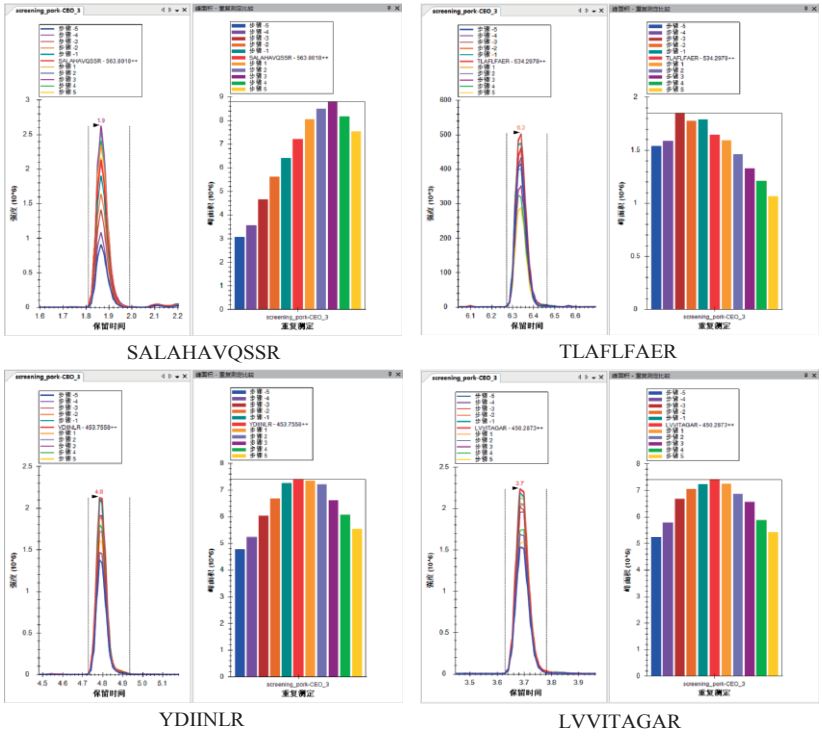


Figure 2. CE energy optimizing with Skyline (characteristic peptides for pork)

Qualitative and quantitative analysis of pork in beef food with LC-MS/MS

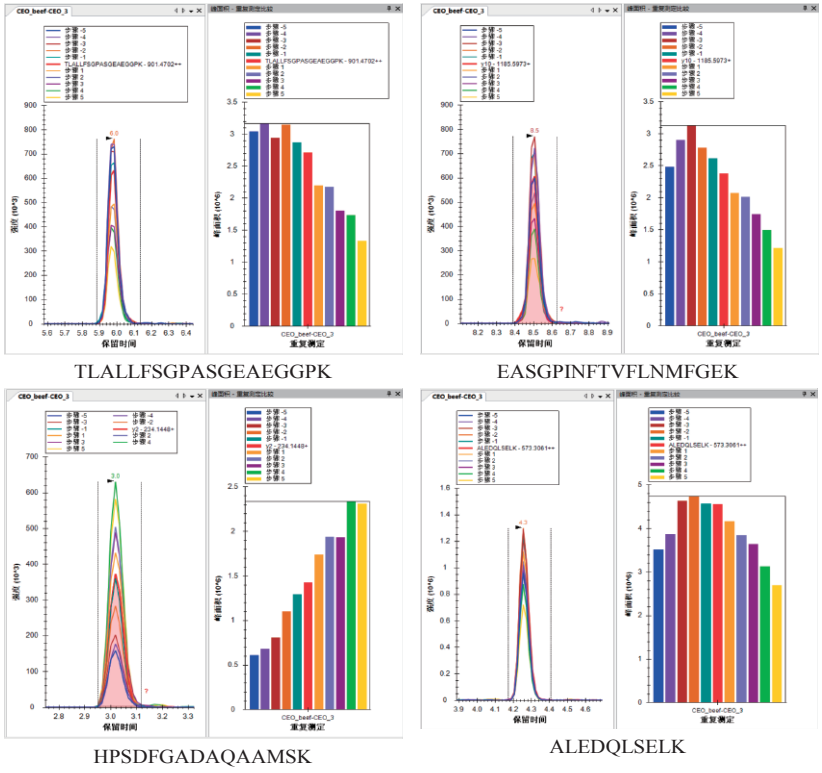


Figure 3. CE energy optimizing with Skyline (characteristic peptides for beef)

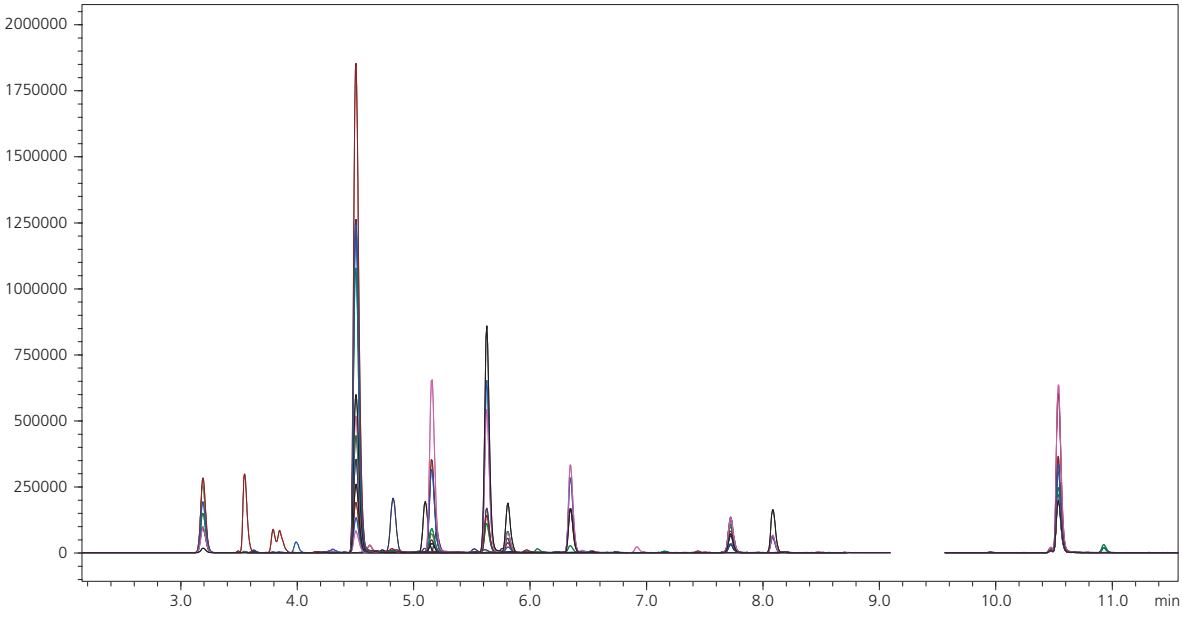


Figure 4. MRM chromatogram of beef with 40% pork in it

Qualitative and quantitative analysis of pork in beef food with LC-MS/MS

Construction of calibration curve for pork and beef

Different amounts of pork and beef were weighed according to the ratio 5/95, 10/90, 20/80, 40/60, 60/40, 80/20, so the calibration curve for the pork was 5%, 10%, 20%, 40%, 60% and 80%, and for the beef was 20%, 40%, 60%, 80%, 90% and 95%. The result indicated that the accuracy for the pork calibration curve was 87.4~110.2%, and for the beef calibration curve was 95.2~104.9%, and the correlation coefficient (r value) is 0.9960 and 0.9982 for the pork and beef calibration curve, respectively.

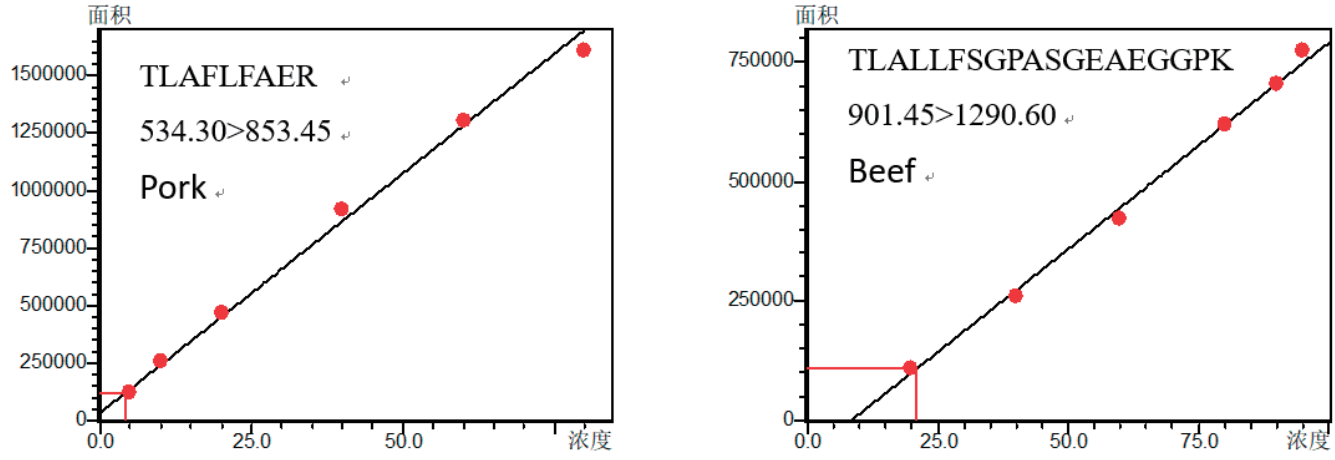


Figure 5. Calibration curve for the characteristic peptides of pork and beef

Meat	Characteristic peptides	Calibration Curve	Linearity range	r	Accuracy (%)
Pork	TLAFLFAER	Y = (9580.83)X + (32460.7)	5~80%	0.9960	87.4~110.2
Beef	TLALLFSGPASGEAEGGPK	Y = (75581.2)X + (452441)	20~95%	0.9982	95.2~104.9

Qualitative and quantitative analysis of pork in beef food with LC-MS/MS

Conclusions

In this paper, 4 characteristic peptides were selected for each kind of meat. The peptides SALAHAVQSSR, TLAFLFAER, YDIINLR and LVVITAGAR were characteristic for pork, and the peptides TLALLFSGPASGEAEGGPK, EASGPINFTVFLNMFGEK, HPSDFGADAQAAMSK, ALEDQLSELK and LVIITAGAR were characteristic for beef. All these peptides were imported to the Skyline software for the ionic transition selection and CE energy optimization. The peptides TLAFLFAER and TLALLFSGPASGEAEGGPK were selected for the quantitative peptides of the pork and beef, and the quantitative transition for these two peptides were m/z 534.30>853.45 and m/z 901.45>1290.60. Different amounts of pork and beef were weighed according to the ratio 5/95, 10/90, 20/80, 40/60, 60/40, 80/20, so the calibration curve for the pork was 5%, 10%, 20%, 40%, 60% and 80%, and for the beef was 20%, 40%, 60%, 80%, 90% and 95%. The result indicated that the accuracy for the pork calibration curve was 87.4~110.2%, and for the beef calibration curve was 95.2~104.9%, and the correlation coefficient (r value) is 0.9960 and 0.9982 for the pork and beef calibration curve, respectively.

First Edition: June, 2019

SHIMADZU

Shimadzu Corporation

www.shimadzu.com/an/

SHIMADZU
Excellence in Science

Application News

No. A628

Fourier Transform Infrared Spectrophotometer (FTIR)

Qualitative Analysis and Quantitative Analysis of Sugars in Maple Syrup by FTIR

Maple syrup is a natural sweetener which is made by boiling down the sap of the sugar maple tree (*Acer saccharum*). Many virgin sugar maple forests, which provide the raw material for maple syrup, grow in southeast Canada and the northeast United States, and the products produced in those regions account for the larger part of distribution.

Food stores handle an abundant range of maple syrups with different colors and tastes to respond to the diverse needs of customers, including products made by mixing corn syrup with maple syrup, as well as low-priced syrup products made by adding the flavor and smell of maple syrup to corn syrup. Corn syrup is a liquid which is produced by using enzymes or acid to decompose corn starch to sugar, and is frequently added to syrup products in the manufacturing process [(1)].

Pure maple syrup consists of various phenols, flavor components, and sugars. Sucrose makes up 90 % or more of the sugars, and the contents of fructose and glucose are less than 2 %. In contrast, glucose is the main component of corn syrup.

In this article, we conducted a qualitative analysis and quantitative analysis of the sugars in maple syrup.

J. Head, J. Kinyanjui, M. Talbott, R. Clifford, R. Fuji

Analysis Method

The Shimadzu IRTracer™-100 Fourier transform infrared spectrophotometer (FTIR) shown in Fig. 1 was used. Measurements were carried out by the 10-bounce attenuated total reflectance (ATR) method using a horizontal ATR accessory (HATR). Table 1 shows the measurement conditions. The maple syrup samples were diluted to 10 % w/w with water and dripped on the ATR prism (ZnSe) for measurement.

Fig. 1 IRTracer™-100

Table 1 Measurement Conditions

Instruments	: IRTracer-100 HATR-10
Resolution	: 4 cm^{-1}
Accumulation	: 32 times
Wavenumber range	: 4000 - 600 cm^{-1}
Apodization function	: Happ-Genzel
Detector	: DLATGS

Qualitative Analysis of Sugars

Fig. 2 shows the infrared (IR) spectra of commercial maple syrup products (pure maple syrup, low-priced maple syrup), corn syrup, and various types of sugars. The IR spectrum of the commercial pure maple syrup, in which sucrose accounts for 98 % of the total content, shows good agreement with the IR spectrum of sucrose, whereas the IR spectrum of the low-priced commercial syrup is consistent with corn syrup. Thus, the quality of maple syrups can be judged quickly by comparison with the IR spectra of corn syrup and various types of sugars.

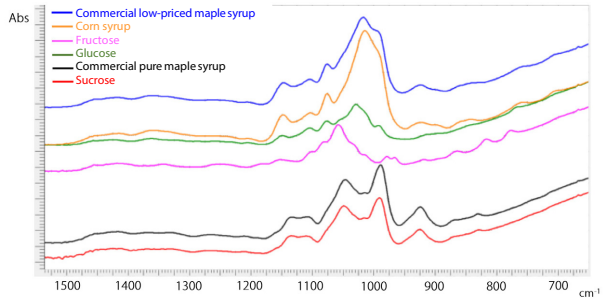


Fig. 2 Infrared Spectra of Commercial Maple Syrups (Pure, Low-Priced), Corn Syrup, and Various Sugars

Quantitative Analysis of Sugars

The concentrations (% w/w) of fructose, glucose, and sucrose in various maple syrups were calculated by chemometrics by the PLS (partial-least squares) method. This analytical method makes it possible to obtain the amounts of target substances in multicomponent mixtures.

Table 2 shows the results of a quantitative analysis of fructose, glucose, and sucrose in various types of maple syrup products. In the commercial pure maple syrups, sucrose accounts for 90 % or more of the total, whereas the concentration of glucose is clearly higher in the commercial low-priced maple syrups. This suggests that the commercial low-priced maple syrups are essentially corn syrup of which glucose is the main component. Moreover, differences in the concentration of fructose can also be seen, depending on the sample.

Table 2 Quantitative Analysis Results of Concentrations of Fructose, Glucose, and Sucrose in Various Maple Syrup Products

Type of Maple syrup/sample ID	Sample name	Fructose % (w/w)	Glucose % (w/w)	Sucrose % (w/w)
Vermont Sampling Kit				
1	Vermont Fancy	3.30 %	0 %	96.70 %
2	Grade A Medium Amber	2.90 %	0 %	97.10 %
3	Grade A Dark Amber	3.70 %	0 %	96.30 %
4	Grade B	6.90 %	0 %	93.10 %
Commercial pure maple syrup				
5	U.S. Grade A Light Amber	0 %	0 %	100 %
6	Grade A Medium Amber	0 %	0 %	100 %
7	U.S. Grade A Dark Amber	3.20 %	0 %	96.80 %
8	U.S. Grade A Dark Amber	5.80 %	0 %	94.20 %
9	U.S. Grade A Dark Amber	8.90 %	0 %	91.10 %
10	Grade A Dark Amber	5.40 %	0 %	95.50 %
11	U.S. Grade A Dark Amber	6.10 %	0 %	93.90 %
12	U.S. Grade A Dark Amber	6.40 %	0 %	93.60 %
13	U.S. Grade A Dark Amber	0 %	0 %	100 %
14	U.S. Grade B	4.50 %	0 %	95.50 %
Commercial low-priced maple syrup				
15	Sample 1	0 %	68.10 %	31.90 %
16	Sample 2	2.80 %	94.40 %	2.80 %
17	Sample 3	8.10 %	87.70 %	4.20 %
18	Sample 4	15.20 %	79.50 %	5.30 %
19	Sample 5	23.40 %	58.90 %	17.70 %

Conclusion

In this article, a qualitative analysis and quantitative analysis of the various types of sugars in maple syrup were conducted by FTIR measurement and chemometric analysis. As results, in comparison with commercial pure maple syrup, the concentration of glucose in commercial low-priced maple syrups was remarkably higher, suggesting that the low-priced products contain corn syrup.

Chemometric analysis enables quantitative analysis of multiple components contained in maple syrup and other food products, and FTIR measurement by the ATR method is extremely simple. Thus, a combination of these techniques is the ideal solution for quality control when qualitative analysis and quantitative analysis of the components of foods is necessary.

<Reference>

(1) Nollet, L.M.L. "Handbook of Food Analysis, Methods and Instruments in Applied Food Analysis". 2nd Edition, Revised and Expanded. Marcel Dekker, Inc. 2004.

*Note: Grading of maple syrups
In 2017, the former standard of 5 grades was revised to a new standard consisting of 4 grades (Golden, Amber, Dark, and Very Dark). The quality of all four ranks is classified as Canadian grade A.

Source:
https://www.maplesource.com/pure-maple-syrup-grades-explained/

The content of this Application News was published by Shimadzu Scientific Instruments in September 2014.

First Edition: Aug. 2020

Shimadzu Corporation
www.shimadzu.com/an/

SHIMADZU
Excellence in Science

Application News

MALDI-TOF Mass Spectrometry Analysis MALDI-8030

Negative Mode Detection of Sulfonated Azo Colourants in Sweets/Candies using the MALDI-8030 Dual Polarity Benchtop MALDI-TOF Mass Spectrometer

S. Salivo (KRATOS ANALYTICAL LTD.)

User Benefits

- Simple easy analysis of sulfonated azo dyes on an affordable benchtop MALDI-TOF
- Quality spectra with good resolution and accuracy in negative ion mode
- Workflow useful for detecting regulated colourants in food

■ Introduction

Synthetic colourants are a type of additive that are widely used in food, pharmaceutical and cosmetics manufacturing. In foods, colourants are used to: i) make food more attractive and appetizing; ii) provide or enhance colours already present; iii) correct natural variations in colour. Among them, sulfonated azo dyes are popular because of their stability, solubility in water and low cost.

Colourants are subject to stringent regulations by different bodies around the world over their safety and adverse effects on human health. For example, in the United States (US), the FDA is responsible for the approval of colourants for use in foods, drugs and cosmetics, while in Europe they are regulated by the European Food Safety Authority.

Among the various approved food colourants, there are some which have been flagged following scientific research, over a possible link to attention deficit hyperactivity disorders (ADHD) in children: Sunset Yellow FCF (E110), Tartrazine (E102) and Allura Red AC (E129). While these dyes have not been completely banned in the EU, food manufacturers are required to apply warning labels on products containing the dyes of concern.

Here, we demonstrate the capability of the dual polarity MALDI-8030 benchtop linear MALDI-TOF mass spectrometer to detect the presence of sulfonated azo colourants in commercial sweets/candies, known to contain these colourants (Table 1). The dyes are selectively extracted via ion-pair extraction [1], and analysed in negative ion mode (Fig 1).

Table 1 List of colourants reported in the ingredients of the commercial candy used for this study

Common Name(s)	E number[b]	Status	Colour
Sunset Yellow FCF (FD&C[a] Yellow 6)	E110	Warning labels in EU. Approved in US.	
Tartrazine (FD&C Yellow 5)	E102	Warning labels in EU. Approved in US.	
Allura Red AC (FD&C Red 40)	E129	Warning labels in EU. Approved in US.	
Brilliant Blue FCF (FD&C Blue 1)	E133	Approved in EU and US.	
Indigo carmine (FD&C Blue 2)	E132	Approved in EU and US.	

a: approved for use in foods, drugs and cosmetics by FDA (US).
b: approved for use in foods by the European Food Safety Authority.

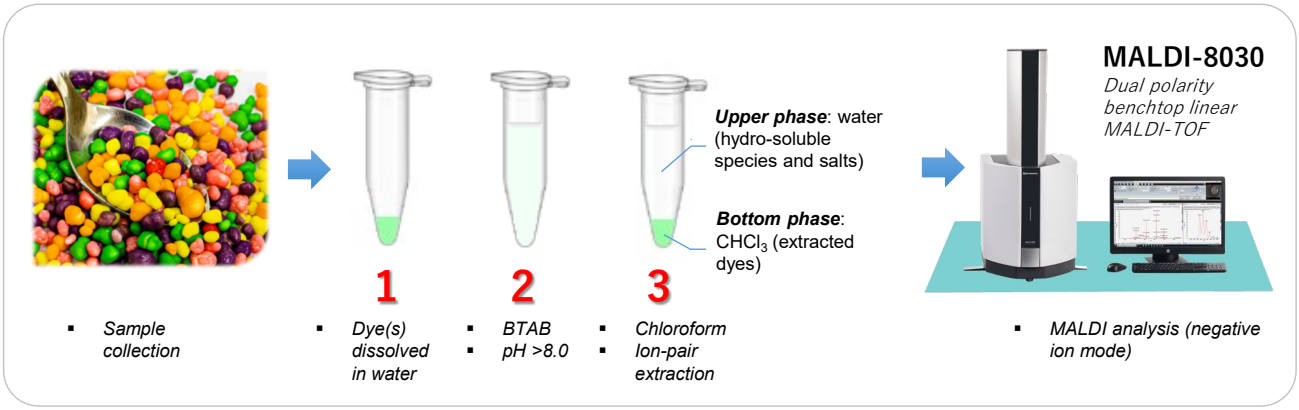


Fig. 1 Sample preparation and analysis workflow for the detection of sulfonated azo colourants in commercial sweets/candies

■ Measurement Conditions and Samples

Samples of US commercial candies were purchased in the UK at a local supermarket. The following sulfonated azo dye standards were purchased from Merck Life Science: Sunset Yellow FCF (dye content 90 %); Allura Red AC (dye content 80 %); Tartrazine (dye content ≥85 %); Brilliant Blue FCF. The reagents used for the ion-pair extraction were: benzyltributylammonium bromide (BTAB) 10 mM [1], Sodium hydroxide (NaOH) 1M for pH adjustment. Individual stock solutions of the dye standards were prepared at 1 mg/mL in 1:1 water/methanol.

The sample preparation workflow is illustrated in Fig 1. The ion-pair extraction method was first optimised and validated with the dye standards. The dyes in the candies were dissolved in water through vortexing until the inner core of the candy was exposed (Fig 1, step 1). Each coloured candy was extracted separately. The aqueous solution containing the dissolved dyes was aspirated and transferred to a microcentrifuge tube, where BTAB (10 mM) was added and the pH adjusted to >8.0 with NaOH (1M) (Fig 1, step 2). Chloroform was then added, and the bi-phasic solution agitated to facilitate the formation of ion-pairs of the sulfonated azo dyes and the subsequent extraction in chloroform (Fig 1, step 3). After centrifugation, the upper (aqueous) phase was discarded, and the bottom (organic) phase containing the extracted dyes recovered for analysis.

For the MALDI analyses, samples were spotted with 9-Aminoacridine (9AA, 10 mg/mL in methanol). All analyses were conducted in negative ion mode on the MALDI-8030.

■ Results of Sulfonated azo dyes (standards)

Fig 2 shows the negative mode MALDI spectra obtained for the sulfonated azo dye standards following ion-pair extraction: Sunset Yellow FCF (FD&C Yellow 6, E110; Fig 2a); Allura Red AC (FD&C Red 40, E129; Fig 2b); Tartrazine (FD&C Yellow 5, E102; Fig 2c); Brilliant Blue FCF (FD&C Blue 1, E133; Fig 2d). The m/z detected correspond to the intact species after removal of the sodium ions as result of the ion-pair extraction. All standard dyes were successfully detected as monoisotopic species along with good mass accuracy: i) Sunset Yellow FCF (m/z 407.001 exact; m/z 407.071 detected; -0.07 Da error); ii) Allura Red AC (m/z 451.027 exact; m/z 451.008 detected; 0.019 Da error); iii) Tartrazine (m/z 466.997 exact; m/z 467.017 detected; -0.02 Da error); iv) Brilliant Blue FCF (m/z 747.151 exact; m/z 747.152 detected; -0.001 Da error).

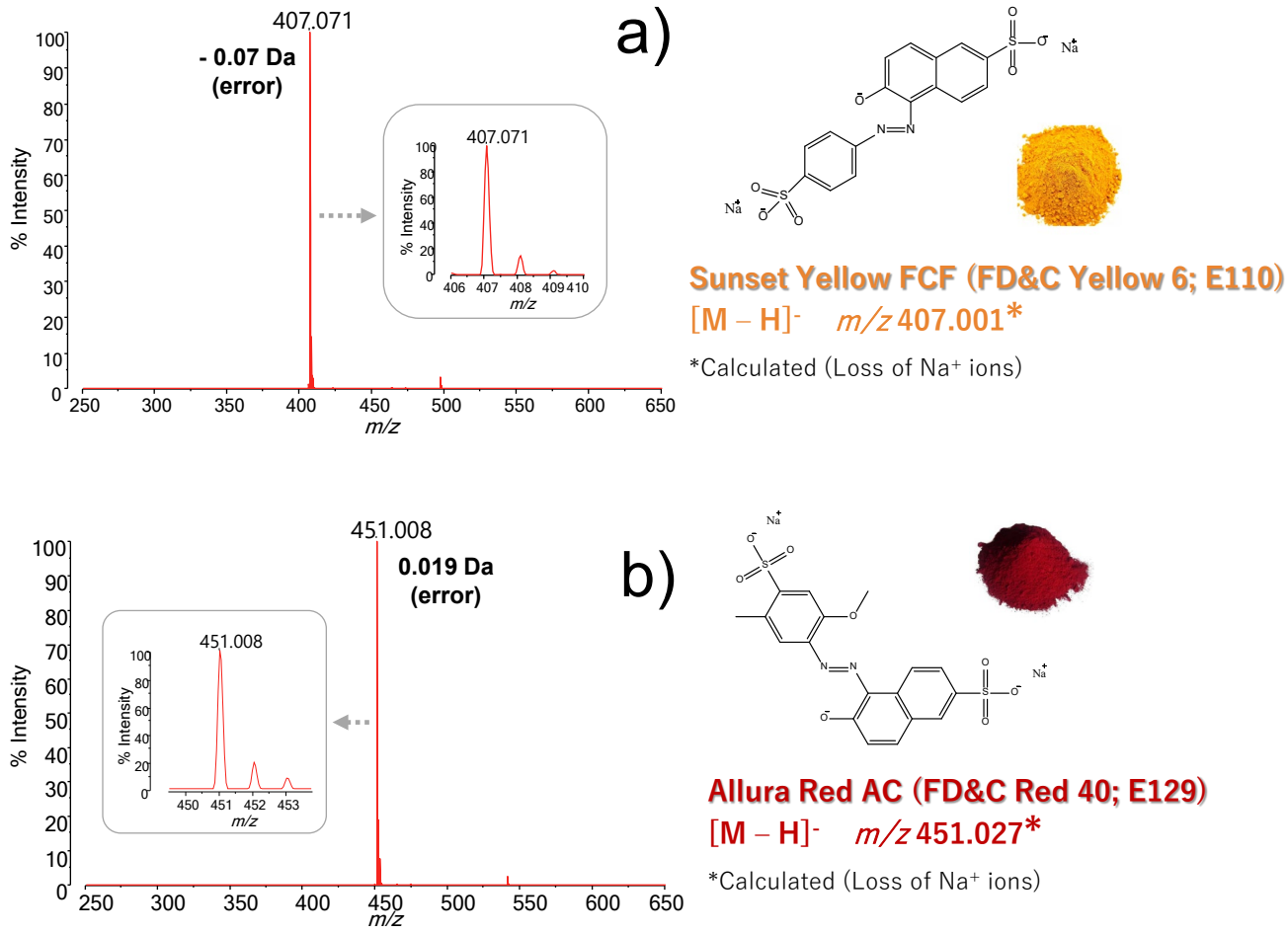


Fig. 2 (continued on next page). Negative mode MALDI spectra of standard sulfonated azo dyes after ion-pair extraction. a) Sunset Yellow FCF (FD&C Yellow 6; E110); b) Allura Red AC (FD&C Red 40; E129)

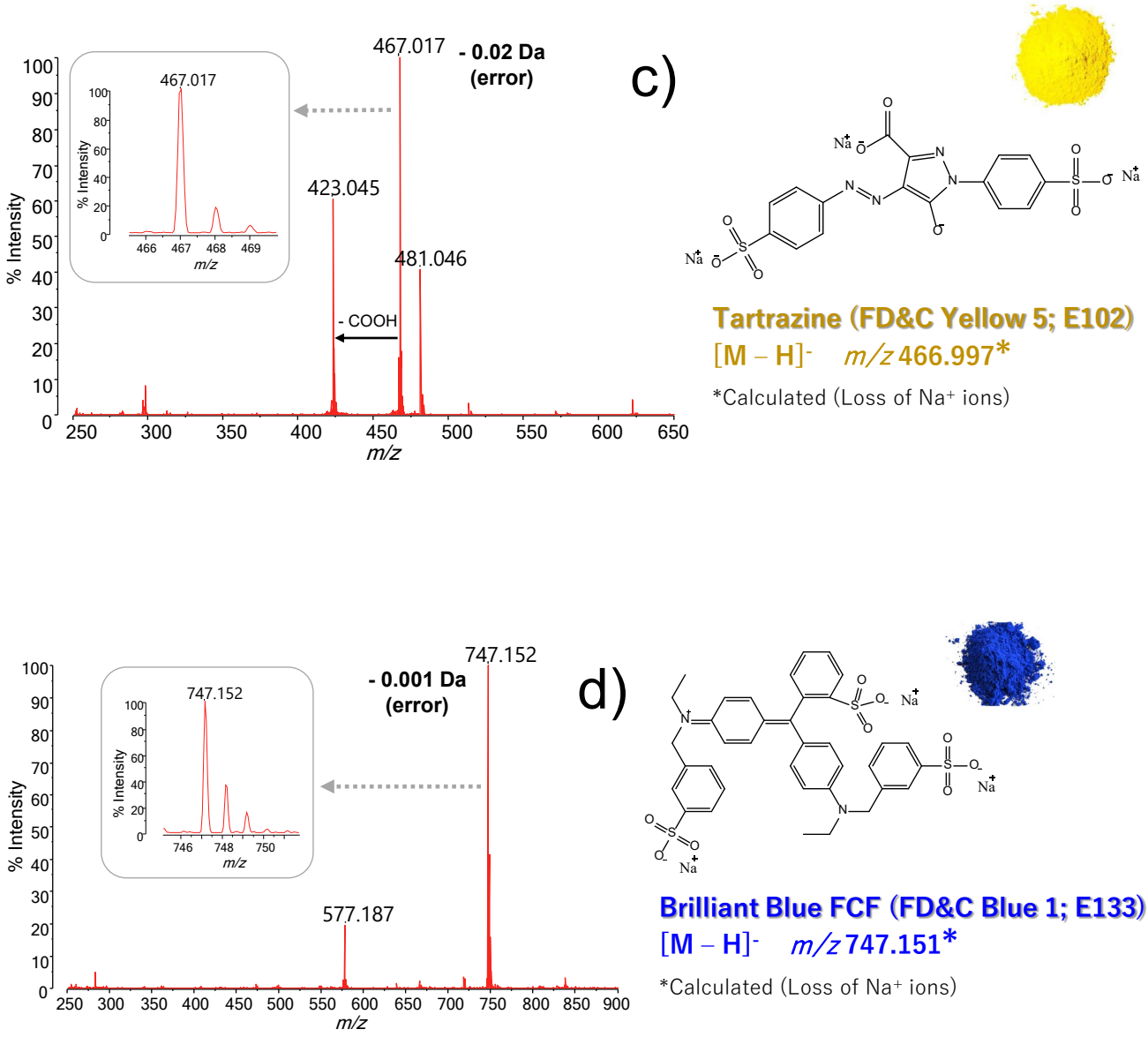


Fig. 2 (continued from previous page). Negative mode MALDI spectra of standard sulfonated azo dyes after ion-pair extraction. c) Tartrazine (FD&C Yellow 5; E102); d) Brilliant Blue FCF (FD&C Blue 1; E133). The exact *m/z* values are calculated for the monoisotopic species after removal of sodium ions. Mass errors (Da) are also provided.

■ Results of Sulfonated azo dyes (candies)

Fig 3 shows the negative mode MALDI spectra obtained for the sulfonated azo dyes extracted from the candies. All dyes found in each candy are consistent with its colour: i) the 'Orange' candy contains the orange Sunset Yellow FCF dye (FD&C Yellow 6, E110); ii) the 'Pink' candy contains the red Allura Red AC dye (FD&C Red 40, E129); iii) the 'Purple' candy contains a combination of Brilliant Blue FCF (FD&C Blue 1, E133) and Indigo carmine (FD&C Blue 2; E132) blue dyes, plus the red Allura Red AC dye (FD&C Red 40, E129); iv) the 'Yellow' candy contains the yellow Tartrazine dye (FD&C Yellow 5, E102); v) the 'Green' candy contains a combination of yellow Tartrazine (FD&C Yellow 5, E102) and blue Brilliant Blue FCF (FD&C Blue 1, E133) dyes.

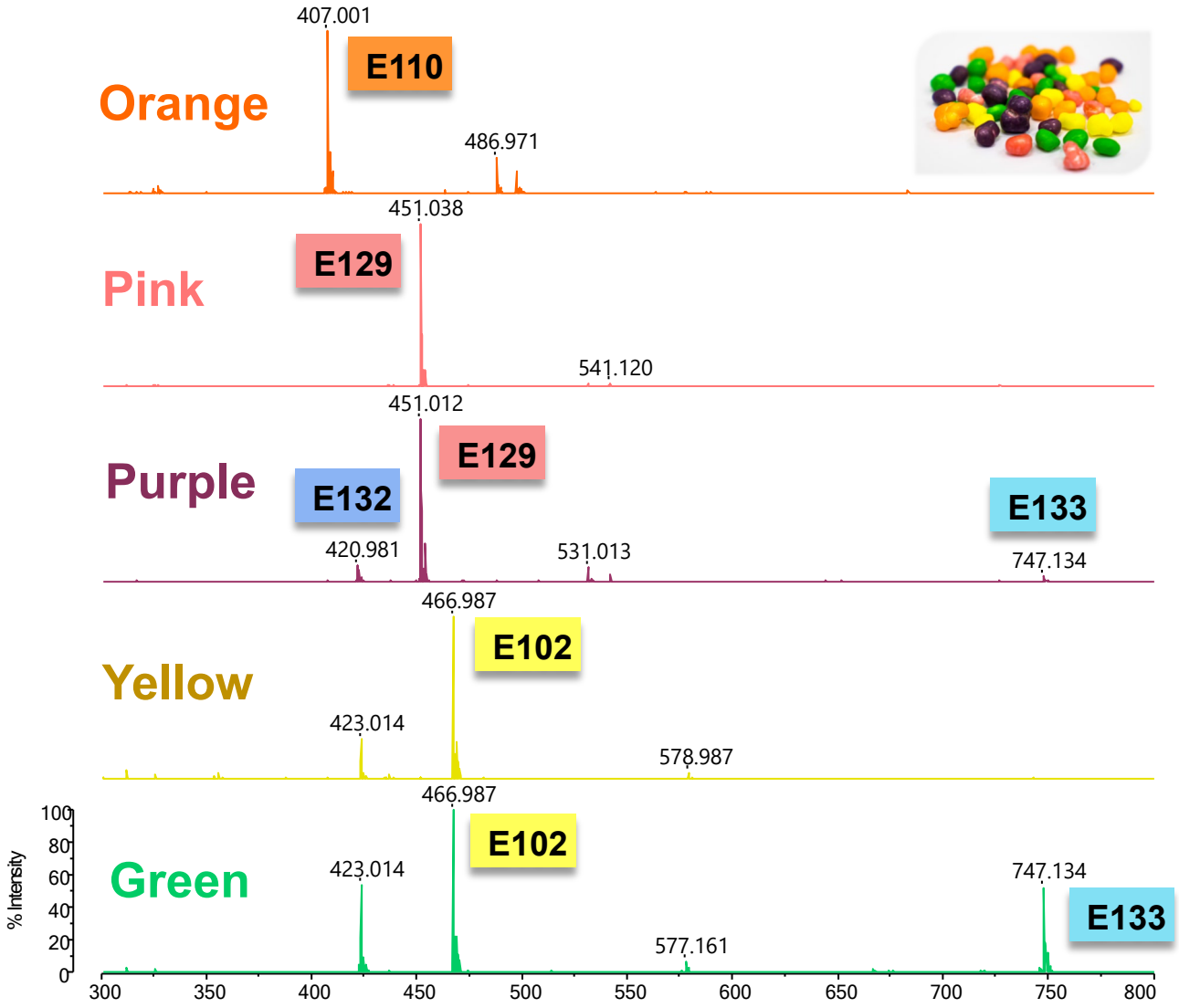


Fig. 3 Negative mode MALDI spectra of sulfonated azo dyes extracted from the commercial candies. The dye content detected with the MALDI analysis is consistent with the colour of the candy.

■ Conclusion

This application demonstrates the capability of the dual polarity MALDI-8030 to detect sulfonated azo colourants in sweets/candies.

The selective extraction method proposed, combined with the negative ion mode detection, offer a simple and fast way to obtain qualitative information on the azo colourant content of confectionery products, which are strictly regulated for their safe use in the food industry.

■ References

[1] Arroyo Negrete, M.A. et al., Anal Bioanal Chem 411, 5833–5843 (2019).

SHIMADZU

Shimadzu Corporation
Analytical & Measuring Instruments Division
Global Application Development Center

www.shimadzu.com/an/

12-MO-483-EN First Edition: Feb. 2022