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Application News

Gas Chromatograph Brevis™ GC-2050

Fatty Acid Analysis of Edible Oils by GC
—Comparing Helium and Hydrogen as Carrier Gases—
—Comparing Analysis by GC with a Simple Screening Analysis by LC-MS—

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User Benefits

- The compact instrument design gives a smaller installation footprint.
- The carrier gas saving mode substantially reduces carrier gas consumption.
- Analysis can be performed using hydrogen as a carrier gas, which is easy to source.
- The single quadrupole LC-MS system can perform simple screening analysis of fatty acids in vegetable oils.

■ Introduction

There are a variety of edible oils that are sold commercially for a range of uses. They are almost entirely composed of compounds called triacylglycerols (neutral lipids), which consist of three fatty acids bound to a glycerol molecule. Because fatty acids affect the functional characteristics of food, determining the fatty acid composition of food is an important area of analysis. In this Application News, the Brevis GC-2050 (GC-FID) is used to determine the fatty acid composition of commercially available edible oils, and the use of hydrogen as a carrier gas is examined and compared with helium. This Application News also analyzes the same edible oils analyzed by LC-MS in a previous Application News[1] and compares the results.

■ Equipment

GC-FID analysis was performed using the Brevis GC-2050 gas chromatograph (Fig.1) , which is designed to be highly compact, with an installation footprint 35 % smaller than the previous model. The Brevis GC-2050 is also equipped with a carrier gas saving mode that reduces carrier gas consumption. The carrier gas saving mode reduced the use of helium carrier gas by around 89 % and hydrogen carrier gas by around 79 % in the analyses described in this article. Hydrogen carrier gas also reduced analysis times by around 25 %.

Fig. 1 Brevis™ GC-2050

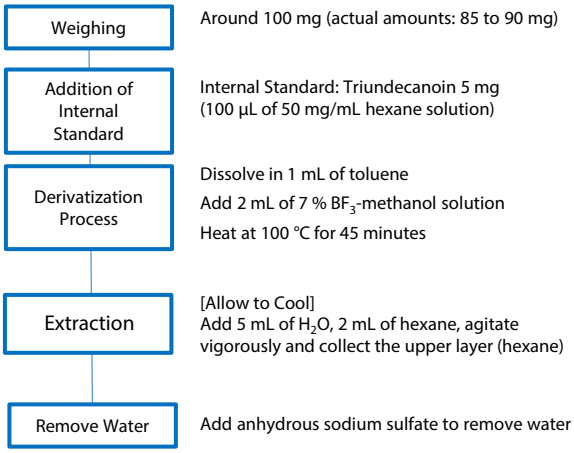

Fig. 2 Sample Preparation Procedure

■ Analysis Conditions

Analysis was performed with both hydrogen and helium as the carrier gas. Table 1 shows the analysis conditions for helium and Table 2 for hydrogen.

Table 1 Analysis Conditions (Helium Carrier)

Model	: Brevis GC-2050
Autosampler	: AOC-30i
Injection Volume	: 1 µL
[GC-2050]	
Injection Mode	: Split
Split Ratio	: 200
Carrier Gas	: He
Carrier Gas Control	: Constant Flow (1.0 mL/min)
Column	: SH-2560 (P/N 227-36311-01) (100 m × 0.25 mm I.D., 0.20 µm)
Column Temp.	: 100 °C (4 min) – 3 °C/min – 240 °C (15 min)
Injector Temp.	: 250 °C
Detector Temp.	: 250 °C

Table 2 Analysis Conditions (Hydrogen Carrier)

Model	: Brevis GC-2050
Autosampler	: AOC-30i
Injection Volume	: 1 µL
[GC-2050]	
Injection Mode	: Split
Split Ratio	:200
Carrier Gas	: H_2
Carrier Gas Control:	: Constant Linear Velocity (25 cm/s)
Column:	: SH-2560 (P/N 227-36311-01) (100 m × 0.25 mm I.D., 0.20 µm)
Column Temp.	: 120 °C (2 min) – 4 °C/min – 220 °C – 2 °C/min – 240 °C (15 min)
Injector Temp.	: 250 °C
Detector Temp.	: 250 °C

■ Sample Preparation

Fatty acid analysis of edible oils by GC requires the derivatization of the fatty acids into fatty acid methyl esters. In this case, derivatization was performed using AOAC Official Method 996.06,[2] excluding the extraction step. The procedure used to prepare samples for analysis is shown in Fig. 2.

■ Results from Calibration Standard Analysis

A commercially available mixture of 37 fatty acid methyl esters (CRM47885, Merck) was analyzed and used to identify fatty acid methyl esters (FAMEs) after sample derivatization. Fig. 3 shows the chromatograms obtained from analyzing the 37 FAME mixture with helium and hydrogen carrier gases. Good separation was achieved with both gases.

■ Results from Real-World Sample Analysis

Edible oils were derivatized and analyzed using both helium and hydrogen carrier gases. Peaks that could not be identified with the calibration standard (37 FAME mixture) were analyzed by GC-MS to determine their carbon number and degree of unsaturation. (Labeled as isomer.)

Fig. 4 shows the chromatograms (partial) obtained from analyzing linseed oil with helium and hydrogen carrier gases. Similar separation was achieved with both gases.

1 C4:0	11 C15:1	21 C20:0	31 C20:4 (c5, c8, c11, c14)
2 C6:0	12 C16:0	22 C18:3 (c6, c9 ,c12)	32 C23:0
3 C8:0	13 C16:1	23 C20:1 (c11)	33 C22:2 (c13, c16)
4 C10:0	14 C17:0	24 C18:3 (c9, c12 ,c15)	34 C24:0
5 C11:0	15 C17:1	25 C21:0	35 C20:5 (c5, c8, c11, c14, c17)
6 C12:0	16 C18:0	26 C20:2 (c11, c14)	36 C24:1
7 C13:0	17 C18:1 (t9)	27 C22:0	37 C22:6 (c4, c7, c10, c13, c16, c19)
8 C14:0	18 C18:1 (c9)	28 C20:3 (c8, c11, c14)	
9 C14:1	19 C18:2 (t9,t12)	29 C22:1 (c13)	
10 C15:0	20 C18:2 (c9,c12)	30 C20:3 (c11, c14, c17)	

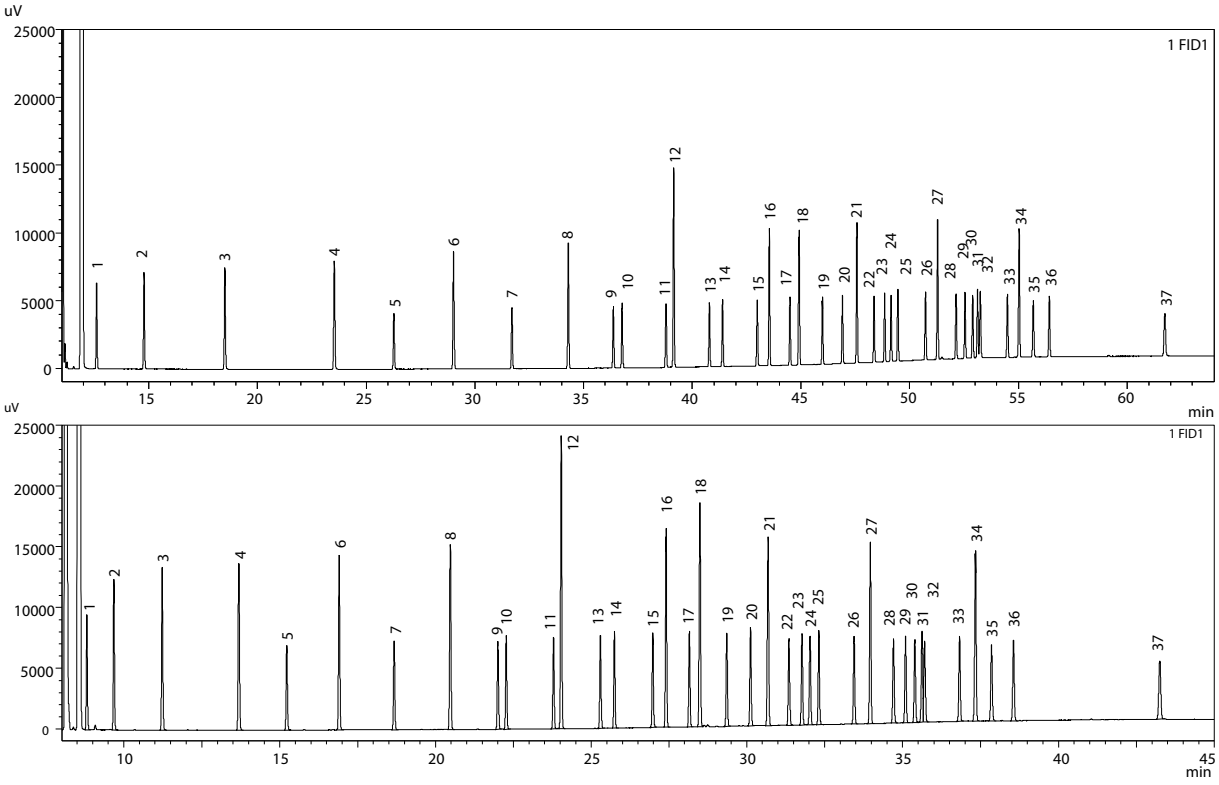

Fig. 3 Chromatograms of Calibration Standard (37 FAME Mixture) (Top: Helium Carrier, Bottom: Hydrogen Carrier)

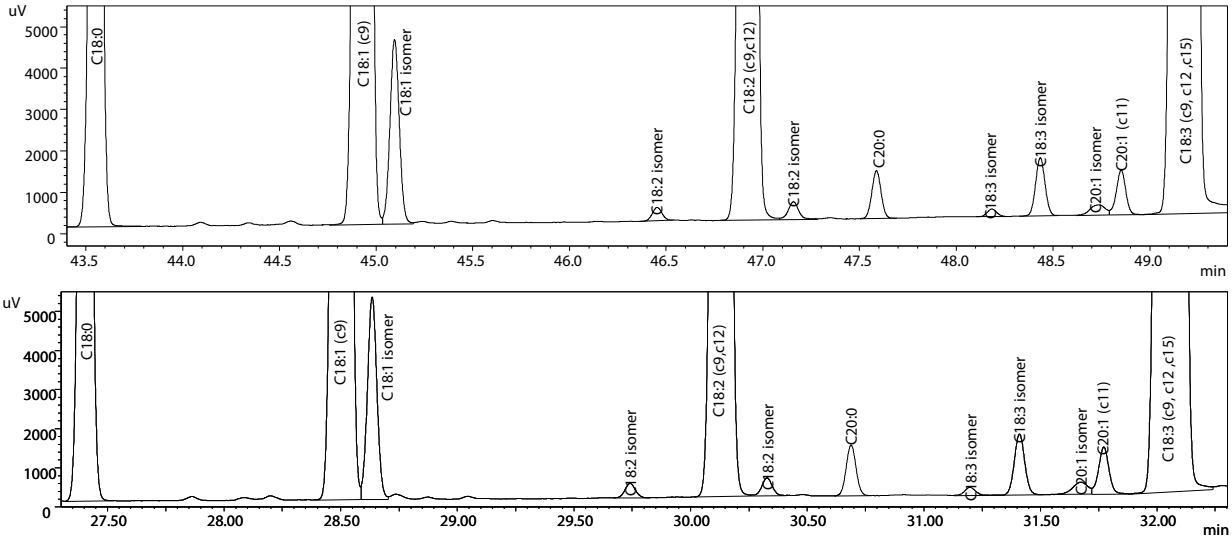

Fig. 4 Chromatograms of Linseed Oil (Top: Helium Carrier, Bottom: Hydrogen Carrier)

Table 3 Fatty Acid Composition (≥ 1 %) of Edible Oils Using Helium Carrier (n = 3, Mean Results)

Sample Name	C8:0 Caprylic acid	C10:0 Capric acid	C12:0 Lauric acid	C14:0 Myristic acid	C16:0 Palmitic acid	C16:1 Palmitoleic acid	C18:0 Stearic acid	C18:1 Oleic acid	C18:1 isomer	C18:2 Linoleic acid	C18:3 Linolenic acid	C20:0 Arachidic acid	C20:1 Eicosenoic acid	C22:0 Behenic acid
Linseed oil 1					4.9 %		4.0 %	14.4 %		15.6 %	**59.0 %**			
Linseed oil 2					5.3 %		4.7 %	20.4 %		15.0 %	**52.3 %**			
Perilla oil					5.2 %		2.2 %	13.8 %		14.0 %	**62.5 %**			
Grape seed oil					6.8 %		4.0 %	18.6 %		**67.5 %**				
Soybean oil					10.2 %		3.6 %	23.2 %	1.4 %	**51.1 %**	6.1 %		1.0 %	
Sesame oil					9.0 %		5.4 %	38.2 %		**44.3 %**				
Macadamia oil					8.0 %	16.3 %	3.3 %	**58.3 %**	3.5 %	2.0 %		2.74 %	2.4 %	
Rice-bran oil 1					12.2 %		1.8 %	**48.9 %**	1.8 %	27.9 %	3.7 %		1.0 %	
Rice-bran oil 2					16.1 %		1.8 %	**42.7 %**	1.0 %	34.1 %	1.0 %			
Rapeseed oil					4.1 %		2.0 %	**58.9 %**	3.3 %	19.6 %	9.2 %		1.0 %	
Olive oil EV					11.8 %		3.7 %	**75.0 %**	2.2 %	4.8 %				
Olive oil					10.0 %		3.2 %	**75.3 %**	1.8 %	7.1 %				
Sunflower seed oil					3.3 %		3.6 %	**81.8 %**		7.9 %				1.0 %
Safflower oil					4.8 %		1.8 %	**77.5 %**		12.9 %				
MCT oil	6.8 %	4.6 %			3.5 %		1.7 %	**55.1** %	2.7 %	15.9 %	6.1 %		1.5 %	
Coconut oil	7.4 %	5.7 %	**46.8 %**	18.0 %	9.4 %		2.9 %	6.9 %		1.8 %				

Table 4 Fatty Acid Composition (≥ 1 %) of Edible Oils Using Hydrogen Carrier (n = 3, Mean Results)

Sample Name	C8:0 Caprylic acid	C10:0 Capric acid	C12:0 Lauric acid	C14:0 Myristic acid	C16:0 Palmitic acid	C16:1 Palmitoleic acid	C18:0 Stearic acid	C18:1 Oleic acid	C18:1 isomer	C18:2 Linoleic acid	C18:3 Linolenic acid	C20:0 Arachidic acid	C20:1 Eicosenoic acid	C22:0 Behenic acid
Linseed oil 1					4.9 %		4.0 %	14.5 %		15.7 %	**59.0 %**			
Linseed oil 2					5.3 %		4.7 %	20.5 %		15.0 %	**52.3 %**			
Perilla oil					5.3 %		2.2 %	13.8 %		14.0 %	**62.4 %**			
Grape seed oil					6.8 %		4.0 %	18.7 %		**67.6 %**				
Soybean oil					10.3 %		3.6 %	23.2 %	1.4 %	**51.1 %**	6.1 %		1.0 %	
Sesame oil					9.0 %		5.5 %	38.2 %		**44.3 %**				
Macadamia oil					8.0 %	16.4 %	3.3 %	**58.2 %**	3.5 %	2.0 %		2.7 %	2.4 %	
Rice-bran oil 1					12.2 %		1.8 %	**48.9 %**	1.8 %	27.9 %	3.6 %		1.0 %	
Rice-bran oil 2					16.2 %		1.8 %	**42.7 %**	0.9 %	34.1 %	1.0 %			
Rapeseed oil					4.1 %		2.0 %	**58.8 %**	3.3 %	19.6 %	9.2 %		1.0 %	
Olive oil EV					11.8 %		3.7 %	**75.0 %**	2.1 %	4.8 %				
Olive oil					10.0 %		3.2 %	**75.3 %**	1.7 %	7.0 %				
Sunflower seed oil					3.3 %		3.6 %	**81.8 %**		7.9 %				1.0 %
Safflower oil					4.9 %		1.8 %	**77.5 %**		12.9 %				
MCT oil	6.6 %	4.5 %			3.5 %		1.7 %	**55.2 %**	2.7 %	16.0 %	6.1 %		1.5 %	
Coconut oil	7.2 %	5.7 %	**46.9 %**	18.1 %	9.5 %		2.9 %	6.9 %		1.8 %				

■ Quantitative Results from Fatty Acid Analysis of Edible Oils

The fatty acid compositions (omitting fatty acids at < 1 %) of 16 commercially sourced edible oils of 13 oil types are shown in Tables 3 and 4. This includes two rice-bran oils and two linseed oils sourced from different producers. The two rice-bran oils from different producers are referred to as rice-bran oil 1 and 2, the linseed oil also analyzed in a previous Application News[1] is referred to as linseed oil 2, and the other linseed oil from a different producer is referred to as linseed oil 1.

Fatty acid compositions were calculated based on FAME peak area as a percentage of the total (after excluding the internal standard C11:0). Edible oils are grouped based on the most abundant fatty acid. The most abundant is shown in red text. The compound referred to as C18:1 isomer was characterized by GC-MS as an isomer of oleic acid. Almost all the fatty acid levels were within 0.1 % when using helium or hydrogen as the carrier gas. The largest difference was 0.2 % (coconut oil: C8:0).

■ Simple Screening Analysis by LC-MS

In the previous Application News,[1] eight commercially available vegetable oils (soybean oil, rapeseed oil, sunflower seed oil, olive oil, grapeseed oil, coconut oil, linseed oil, and perilla oil) were analyzed. Each vegetable oil was diluted 100 times with 2-propanol, and DHA was added during dilution to a final concentration of 10 ppm as an internal standard.

Analysis was performed using a single quadrupole LC-MS system and samples were delivered for MS by flow injection (FIA-MS). Delivering samples by flow injection gives a high throughput of one sample per minute, making it suitable for high-throughput analysis of large numbers of samples. Mass spectrometers are prone to contamination with flow injection, as flow injection because it delivers samples directly to the mass spectrometer without passing through a column. However, the robustness and ease of maintenance of the LCMS-2050 (Fig. 5) make it suitable for flow injection. (See the previous Application News[1] for more information on the analysis conditions.)

Table 5 Comparison of GC-FID (Helium Carrier) and FIA-MS (1/2)

Sample Name	Linseed oil 2		Perilla oil		Soybean oil		Grape seed oil	
Compound name	FIA-MS	GC-FID	FIA-MS	GC-FID	FIA-MS	GC-FID	FIA-MS	GC-FID
Palmitic acid C16:0	4.8 %	5.3 %	4.5 %	5.2 %	8.0 %	10.2 %	6.1 %	6.8 %
Stearic acid C18:0	5.4 %	4.7 %	0.6 %	2.2 %	3.6 %	3.6 %	3.7 %	4.0 %
Oleic acid C18:1	25.9 %	20.4 %	18.5 %	13.8 %	28.5 %	23.2 %	22.2 %	18.6 %
Linolic acid C18:2	18.8 %	15.0 %	18.8 %	14.0 %	48.1 %	51.1 %	60.2 %	67.5 %
Linolenic acid C18:3	45.1 %	52.3 %	57.7 %	62.5 %	11.9 %	6.1 %	7.1 %	0.4 %

Table 6 Comparison of GC-FID (Helium Carrier) and FIA-MS (2/2)

Sample Name	Sunflower seed oil		Olive oil		Rapeseed oil		Coconut oil	
Compound name	FIA-MS	GC-FID	FIA-MS	GC-FID	FIA-MS	GC-FID	FIA-MS	GC-FID
Caprylic acid C8:0							6.0 %	7.4 %
Capric acid C10:0							4.6 %	5.7 %
Lauric acid C12:0							42.7 %	46.8 %
Myristic acid C14:0							21.3 %	18.0 %
Palmitic acid C16:0		3.3 %	8.6 %	10.0 %	3.9 %	4.1 %	8.8 %	9.4 %
Stearic acid C18:0	4.8 %	3.6 %	4.6 %	3.2 %		2.0 %	3.3 %	2.9 %
Oleic acid C18:1	85.9 %	81.8 %	78.1 %	75.3 %	66.7 %	58.9 %	10.8 %	6.9 %
Linolic acid C18:2	9.3 %	7.9 %	8.7 %	7.0 %	20.5 %	19.6 %	2.6 %	1.8 %
Linolenic acid C18:3					8.9 %	9.2 %		

■ Comparing GC-FID and FIA-MS

Tables 5 and 6 compare the results of analyzing eight vegetable oils for fatty acids by FIA-MS (screening analysis) and GC-FID (a helium carrier). The results reveal characteristic features of the vegetable oils, such as the most abundant fatty acids, and that coconut oil contains fatty acids with relatively low carbon numbers. More information about the screening analysis performed with a single quadrupole mass spectrometer can be found in Shimadzu Application News 01-00674-EN.[1)]

Fig. 5 Nexera™ and LCMS-2050 System

■ Conclusion

In this Application News, the Brevis GC-2050 was used to determine the fatty acid composition of edible oils. Fatty acid analysis is typically performed using helium as the carrier gas, but in this study, hydrogen was used. This reduced analysis times while producing results that compared favorably with helium. Given the current supply issues associated with helium, hydrogen should be considered as an alternative to helium.

The results also show that fatty acid compositions determined by FIA-MS correlate well with results obtained by GC-FID. And because the sample pretreatment only requires sample dilution, analysis by FIA-MS is rapid, taking just 1 minute. Therefore, FIA-MS seems particularly well-suited to the screening of large numbers of samples or when there is an urgent need to verify quality or safety.

<References>

1) Shimadzu Application News: Simple Method for Screening Analysis of Vegetable Oils Using a Single Quadrupole Mass Spectrometer, 01-00674-EN

2) AOAC Official Method 996.06 Fat (Total, Saturated, and Unsaturated) in Foods: Hydrolytic Extraction Gas Chromatographic Method

01-00852-EN First Edition: Aug. 2025

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