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GCMS – Automation – Food Quality And Nutrition

Automation Of FAME Derivatization For Fatty Acid Profiling In Various Edible Oils Using AOC-6000 Plus

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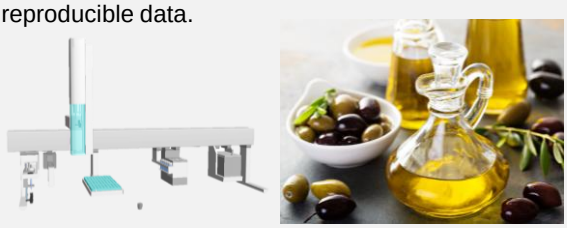
Abstract

To perform fatty acid profiling in edible oils using GCMS, derivatization to fatty acid methyl esters (FAME) is required. However, conventional derivatization can be laborious, time-consuming, and generate significant chemical waste.

In this Technology Brief, we present a solution utilizing AOC-6000 Plus, which fully automates the sample preparation steps. This automation can free up valuable man-hours and reduce operator workload, ultimately contributing to higher productivity. Furthermore, the automated workflow significantly reduces the scale of the experiment without compromising data quality, leading to a significant reduction in waste. This improvement enhances both the sustainability and efficiency of laboratory processes.

Lastly, in the automated workflow, all samples are freshly prepared in an identical manner and injected into the GCMS almost immediately once ready, enabling highly reliable and reproducible data.



Enabling walkaway automation in edible oil analysis.

Keywords:
Automation, Edible Oil, Fatty Acid Profiling, FAME Derivatization

Highlights

- *Full Automation of edible oil sample preparation for fatty acid profiling.*
- *All samples are freshly prepared and injected into GCMS in an identical manner.*
- *Highly robust, reproducible, and reliable results obtained due to automation.*

Technologies Featured

Automated Sample Prep with AOC-6000 Plus

GCMS-QP2020 NX

1. INTRODUCTION

Industrial processes, such as refining and high-temperature treatments, are used to alter the physical properties of edible oils (e.g., deodorizing, bleaching, and hydrogenation) to make them more suitable for broader applications [1]. However, such high-temperature processes can potentially impact the chemical composition of fatty acid profiles, leading to the formation of undesired chemical species like trans-fats [1]. To address this issue, several regional regulations have been established to set limits on the acceptable levels of trans-fats in edible oil products and to ensure proper reporting of nutritional information.

By incorporating fatty acid profiling into their quality control procedures, manufacturers can comply with the legal requirements set by different regional authorities and provide consumers with transparent information, helping them to make healthier dietary choices. Fatty acid profiling is also essential for assessing the authenticity of edible oils and determining their sources of origin.

In edible oils, fatty acids exist in the forms of free fatty acids and triglycerides (esters consisting of three fatty acids linked to glycerol). Analyzing free fatty acids and triglycerides using GCMS can be challenging due to their low volatility. Thus, they need to be derivatized to fatty acid methyl esters (FAME) prior to GCMS analysis. Herein, we will demonstrate a fully automated process for preparing samples to derivatize the free fatty acids and triglycerides in various edible oils into FAME using AOC-6000 Plus. The FAME profiles obtained in this study effectively represent the fatty acid profiles present in the edible oils.

2. PRINCIPLES BEHIND DERIVATIZATION WORKFLOW

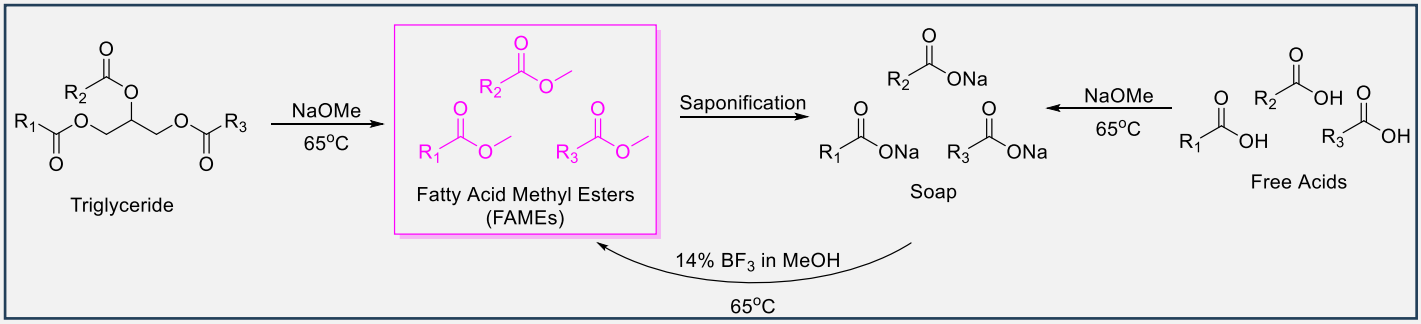


Fig. 1 Reaction scheme of the different chemical species upon addition of NaOMe and BF_3.

Upon the addition of NaOMe (NaOH dissolved in MeOH) to the edible oil sample at 65°C, triglycerides will be hydrolyzed to FAME as depicted in **Fig. 1**. It is challenging to precisely stop the reaction at the conversion to FAME, and saponification will occur upon further incubation (**Fig. 1**). Free fatty acids that are already present in the sample will also be neutralized to form soap species. A strong Lewis acid, BF_3, is added to catalyze the conversion of all side products (soap) to FAME before injection to GCMS.

3. EXPERIMENT

3.1 Reagents Preparation

0.5M NaOMe

Weigh 2 grams (±0.1 gram) of NaOH (Merck V800383) and transfer it to a 250 mL container. Add 100 mL of MeOH (Kanto Chemical, 25183-4B, for pesticide residue and PCB tests) and sonicate for approximately an hour to ensure complete dissolution. White precipitate (sodium carbonate) may be observed to form at the bottom of the container (over time) due to reaction with atmospheric CO_2; this can be ignored.

Saturated NaCl

Weigh 40 grams (±0.1 gram) of NaCl (Merck 31434) and transfer it to a 250 mL container. Add 100 mL of ultrapure water to dissolve the salt and sonicate for approximately an hour. Add approximately one more spoonful of NaCl to the solution to ensure it remains saturated throughout the experiment.

14% BF_3 in MeOH and Hexane

14% BF_3 in MeOH (Merck B1252) and hexane (Kanto Chemical, 18041-3B, for pesticide residue and PCB tests) were purchased commercially. Transfer approximately 10 mL of each prepared reagent (NaOMe, Saturated NaCl, and 14% BF_3 in MeOH) to separate clean 10-mL vials and place them in the Standard Wash Modules in the AOC-6000 Plus autosampler.

3.2 Measurement Conditions And Samples Handling Before Automation

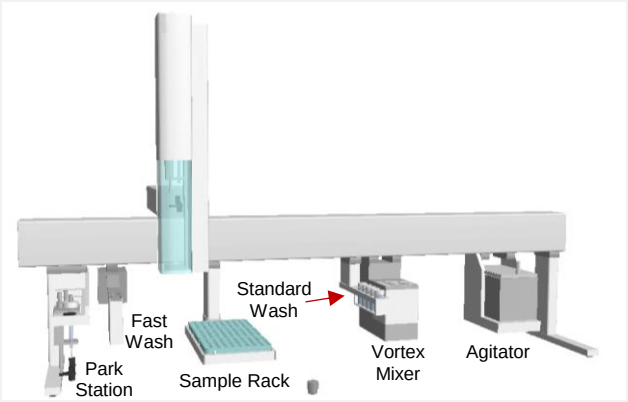


Fig. 2 AOC-6000 Plus modules for automation of FAME derivatization

Sample preparation and injection were performed using AOC-6000 Plus autosampler (modules necessary for automation of FAME derivatization are shown in **Fig. 2**). GCMS-QP2020 NX was utilized to carry out the analysis. Analytical conditions used are depicted in **Table 1**.

Table 1. GCMS Configuration and Parameters

Instrument	GCMS-QP2020 NX
Autosampler	AOC-6000 Plus with PAL Method Composer Software
Flow Control Mode	Linear Velocity
Linear Velocity	20 cm/s
Injection Mode	Split (Split Ratio = 100)
Injection Port Temp.	225°C
Carrier Gas	He
Column	SH-2330 (105 m length x 0.25 mm I.D. x 0.25 µm df) P/N: 227-36239-03
Interface Temp.	250 °C
Solvent Cut Time	11 min
Acquisition Mode	Scan m/z 35 to 500

Fully Automated Workflow

AOC-6000 Plus with PAL Method Composer software enables simple customization of automated derivatization workflow to perform the steps described in **Fig. 3** without the need for any programming background. Syringe washing with IPA and/or ultrapure water is included between each of the steps when necessary. No human/manual intervention is required.

Edible Oil Samples To Be Analyzed

Transfer 10 (±0.5) mg of the edible oil sample into a 1.5-mL vial (P/NL 226-54110-11). Cap the sample vial with a magnetic screw cap (P/N: 226-84128-01).

The amount of oil sample to be transferred can be further reduced to 5 (±0.5) mg if the MS detector is observed to be constantly saturated.

3.3 Automation Of Sample Preparation By AOC-6000 Plus

Hydrolysis
- Transfer 150 µL of 0.5M NaOMe to the oil sample in the sample vial using a 1000-µL syringe (**Fig. 4a**).
- Transfer the sample vial to the agitator and incubate at 65°C, 300 rpm for 10 minutes (**Fig 4b**).
- Return the vial to the sample rack.

Esterification
- Transfer 150 µL of 14% BF_3 in MeOH to the sample vial.
- Transfer the sample vial to the agitator and incubate it at 65°C, 300 rpm for 10 minutes (**Fig. 4b**).
- Return the vial to the sample rack.

Extraction
- Transfer 600 µL of hexane to the sample vial.
- Transfer 150 µL of saturated NaCl solution to the sample vial.
- Transfer the sample vial to the vortex mixer and mix it at 1200 rpm for 20 seconds (**Fig. 4c**).
- Return the vial to the sample rack and wait for 1 minute and 30 seconds to allow for phase separation.

Injection
- Transfer 150 µL of the supernatant into a clean vial (**Fig. 4d**).
- Park the 1000-µL syringe and take a 10-µL syringe (**Fig. 4e**).
- Injection into the GCMS (**Fig. 4f**).

Fig. 3 Workflow of FAME derivatization steps performed by AOC-6000 Plus

Reagent vial positions

1. 0.5M NaOMe
2. 14% BF_3 in MeOH
3. Hexane
4. NaCl (saturated)
5. Waste

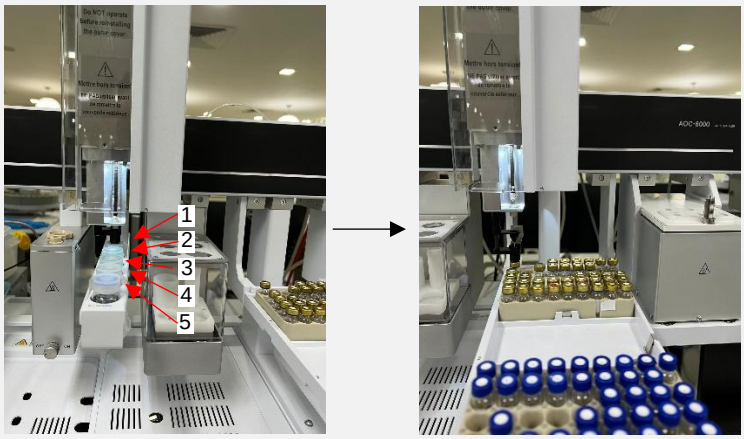


Fig. 4a Transferring 150 µL of 0.5M NaOMe from the 10-mL reagent vial (position 1) to the sample vial.

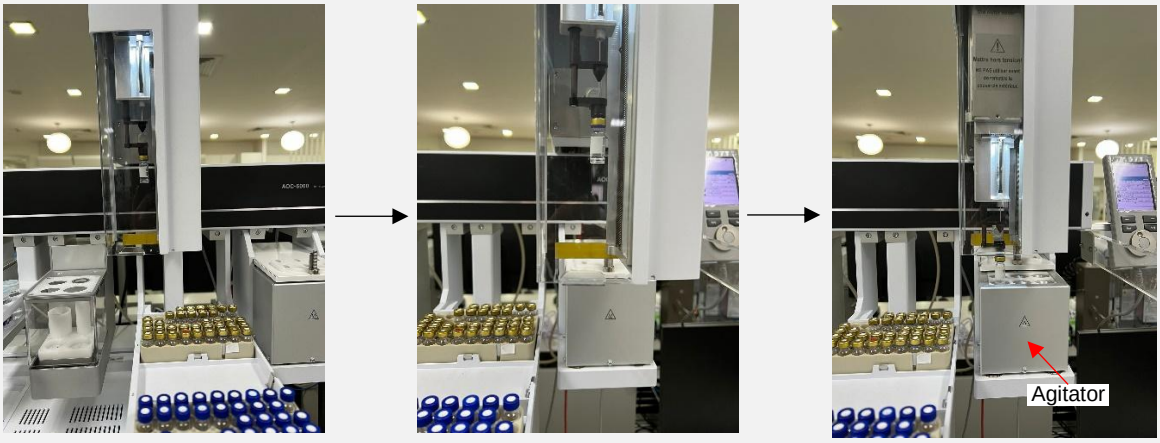


Fig. 4b Transferring the sample vial to the agitator (temperature: 65ºC at 300 rpm) for incubation.

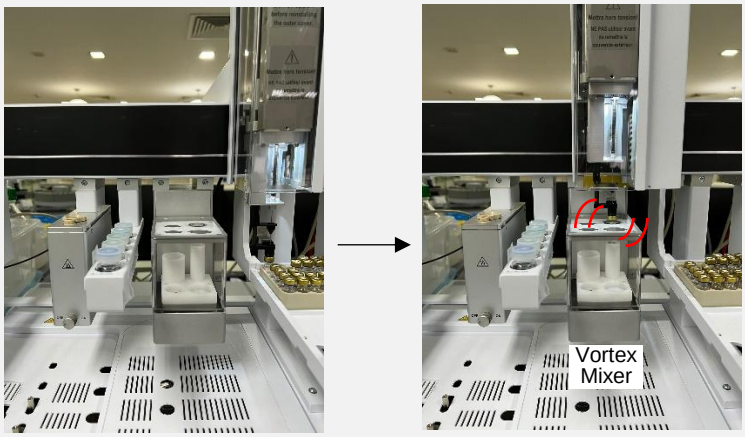
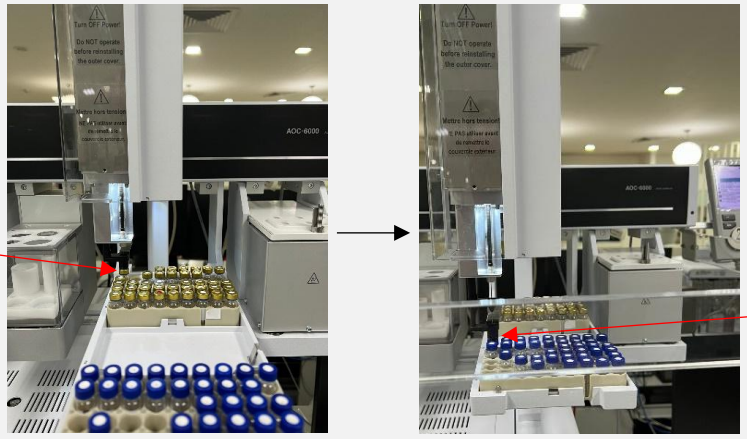


Fig. 4c Transferring the sample vial to the vortex mixer for high speed vortexing (1200 rpm).

Sample vial where phase separation took place.



A new clean vial to contain the supernatant after phase separation.

Fig. 4d Transferring 150 µL of supernatant (after phase separation) from sample vial to a new clean vial to prepare for GCMS injection.

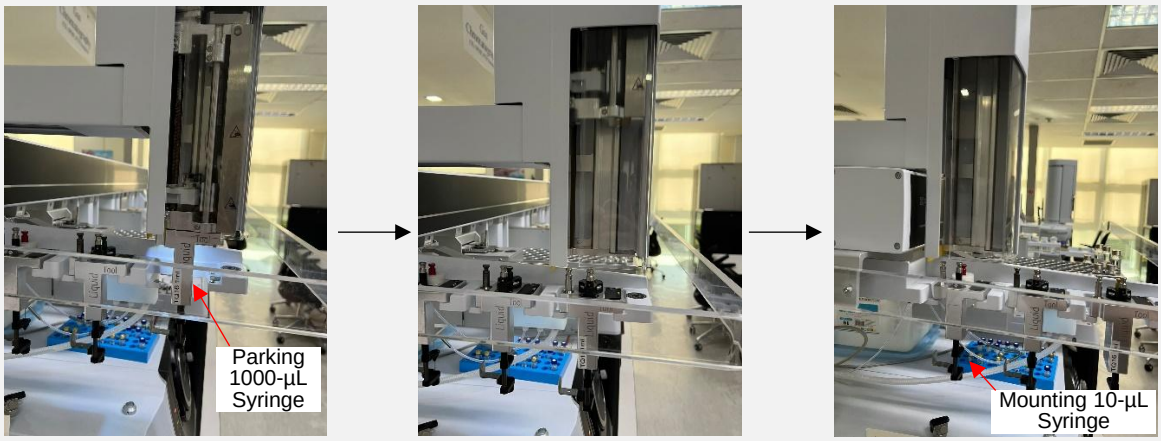


Fig. 4e Changing of the 1000-µL syringe to a 10-µL syringe to prepare for GCMS injection.

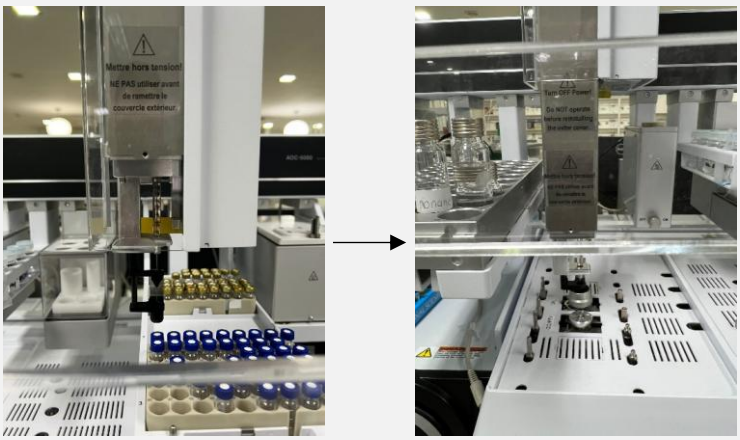


Fig. 4f Drawing sample and subsequent injection into GCMS using the a 10-µL syringe.

4. RESULTS AND DISCUSSION

4.1 Drastic Reduction In The Reagents Required

Table 2 provides an overview of the reagent amounts used in this Technology Brief compared to the conventional AOAC 969.33 method, which has a very similar experimental protocol [2]. Due to higher method efficiency, there is a drastic reduction in the amount of chemicals required for this proposed workflow. As such, less waste is generated compared to conventional approach [3]. The implementation of the proposed automation workflow will encourage greater sustainability in the workplace [3].

Table 2. Amount of Sample and Reagents Required (AOAC 969.33 vs Current Technology Brief) [2]

Sample/Reagents	Amount of Sample and Reagents Required	
	AOAC 969.33[2]	Current Technology Brief
Edible Oil Sample	100 mg	10 mg
0.5M NaOMe	4 mL	150 µL
14% BF_3 in MeOH	5 mL	150 µL
Heptane	5 mL	NA
Hexane	NA	600 µL
NaCl (Saturated)	15 mL to 20 mL	150 µL

4.2 Data Obtained Agrees With Literatures

The 13 edible oil samples were purchased from commercial supermarkets. The results for all samples, summarised in **Table 3-8**, agree with the results from literatures. All the data presented in **Tables 3-8** are rounded off to the nearest percentage. Thus, FAME species detected below 0.5% are rounded down to 0.

Results of Palm Oil Samples (Brand A-D)

Comparing the total ion chromatograms (TIC) results of all palm oil samples (**Fig. 5a-d** and **Table 3**), it was observed that the samples exhibit very similar FAME profiles despite the differences in their physical appearance (e.g., red palm oil vs palm oil). All palm oil samples contain methyl oleate as the most abundant peak (43% to 46%), followed by methyl palmitate (33% to 37%) and methyl linoleate (10% to 14%). The results obtained are very close to the reported literature values [4]. However, red palm oil brand B stands out as it contains around 4% methyl laurate which is not observed in the rest of the samples.

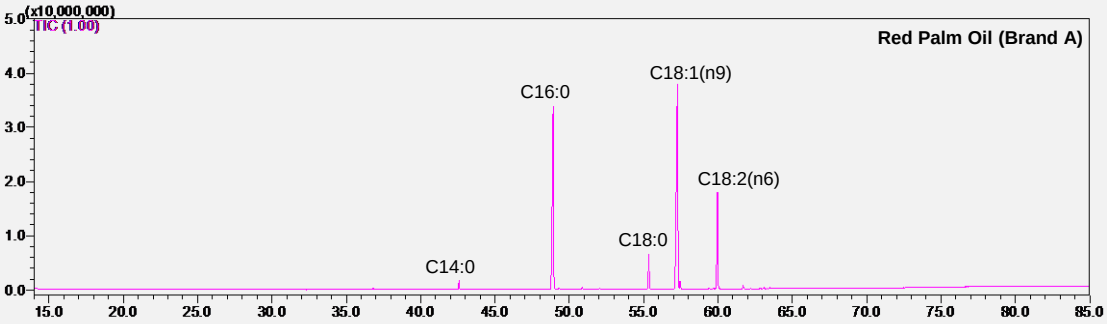


Fig. 5a FAME profile of red palm oil (Brand A)

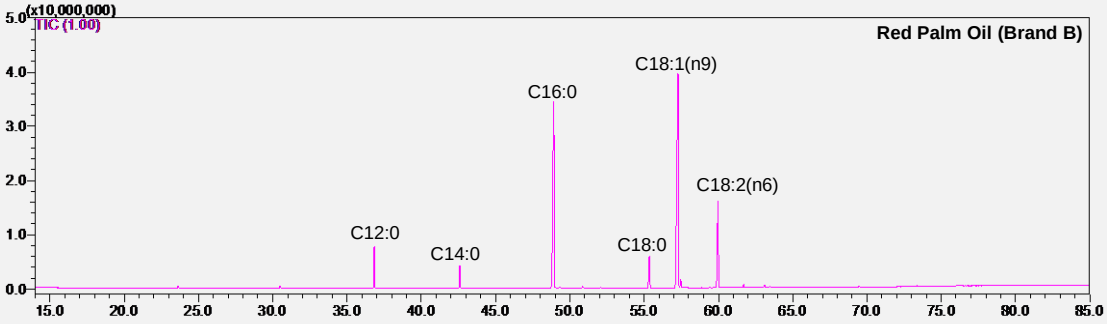


Fig. 5b FAME profile of red palm oil (Brand B)

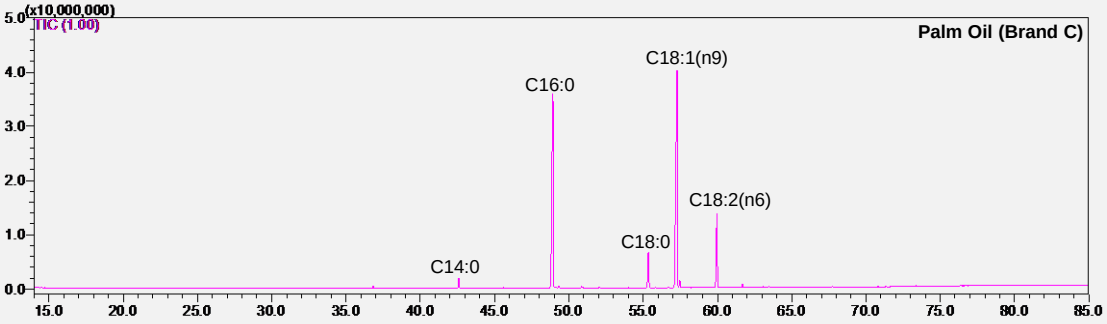


Fig. 5c FAME profile of red palm oil (Brand C)

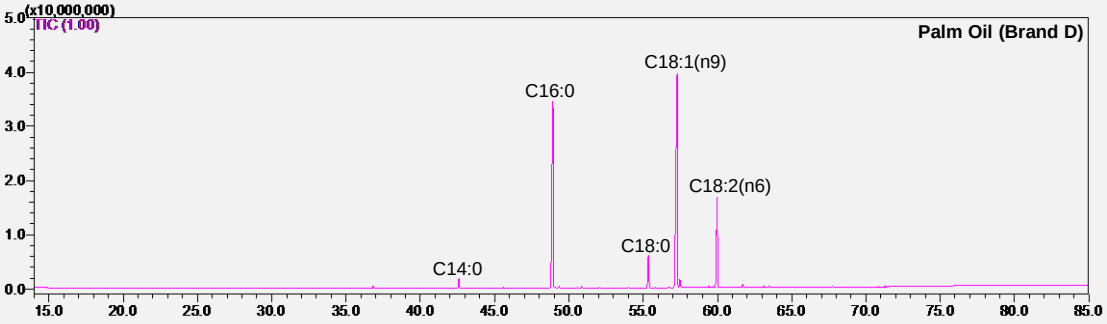


Fig. 5d FAME profile of red palm oil (Brand D)

Table 3. Summary of the FAME Profiles of Palm Oil Samples (TIC Area%) and its Literature Reference Values (FID Area%) [4]

Fatty Acid Methyl Ester	Red Palm Oil (Brand A) /Area%	Red Palm Oil (Brand B) /Area%	Palm Oil (Brand C) /Area%	Palm Oil (Brand D) /Area%	Reference Value[4] /Area%
Methyl Laurate, C12:0	0	4	0	0	0
Methyl Myristate, C14:0	1	2	1	1	1
Methyl Palmitate, C16:0	35	33	37	35	37
Methyl Stearate, C18:0	5	4	5	4	4
Methyl Oleate, C18:1 (n9)	44	43	46	46	45
Methyl Linoleate, C18:2 (n6)	14	12	10	13	12

All the data in the table above are rounded off to the nearest percentage.

Results of Coconut Oil Samples (Brand E-G)

Fig. 6a-c depicts the TIC of the coconut oil samples used in the experiment, and **Table 4** summarizes the results in Area% for the FAME profiles detected. All three chromatograms (**Fig. 6a-c**) obtained are highly identical to one another, and the data obtained agree with the reported values found in the literature [5]. Slight variations are likely attributed to the sources/species of the coconut used, harvesting protocols, and differences in extraction processes [5].

One distinct feature observed for the FAME profiles of coconut samples is the presence of shorter chain FAME, mainly methyl caproate (~1%), methyl caprylate (7% to 8%), and methyl caprate (6% to 7%). Typically, these shorter-chain FAME are not found above 1% in other edible oil samples discussed in this Technology Brief, making them a distinctive feature of coconut oil.

Methyl Laurate (41% to 47%) is observed to be the major FAME in coconut oil followed by methyl myristate at around 19% to 20%. This is very different from the rest of the edible oils (excluding mustard oil), whereby most of them have methyl oleate as the major FAME species.

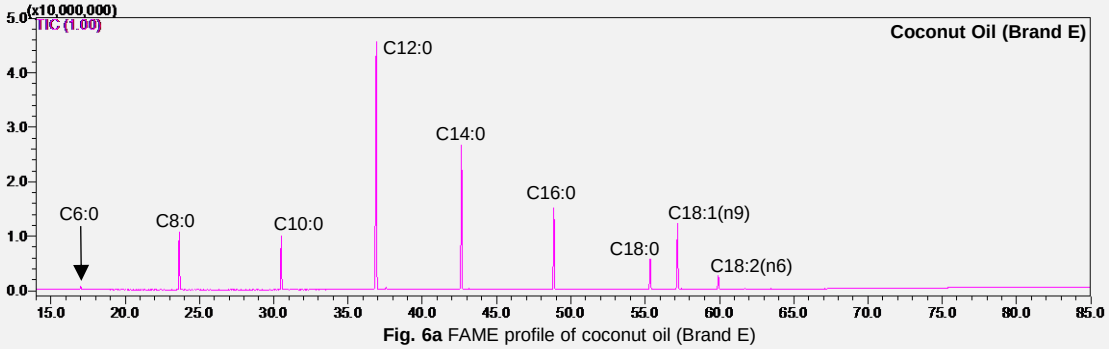


Fig. 6a FAME profile of coconut oil (Brand E)

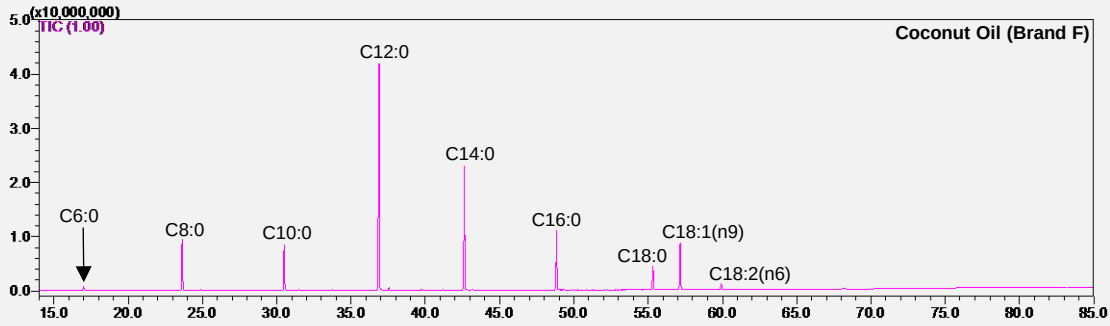


Fig. 6b FAME profile of coconut oil (Brand F)

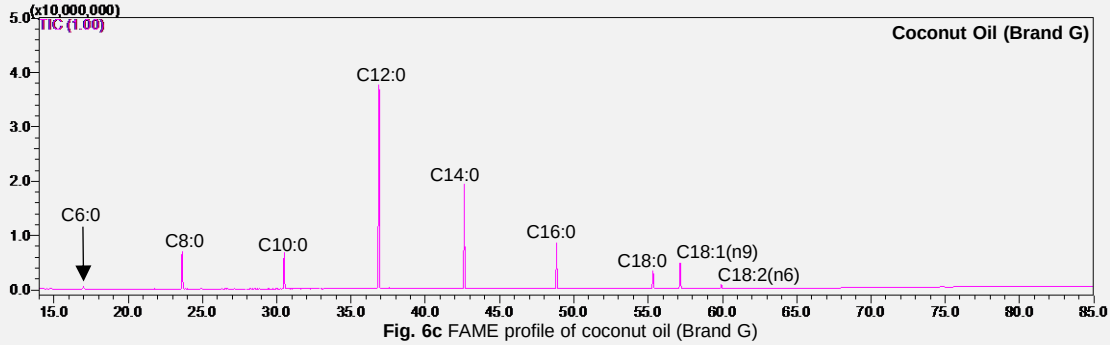


Fig. 6c FAME profile of coconut oil (Brand G)

Table 4. Summary of the FAME Profiles of Coconut Oil Samples (TIC Area%) and Its Literature Reference Values (FID Area%) [5]

Fatty Acid Methyl Ester	Coconut Oil (Brand E) /Area%	Coconut Oil (Brand F) /Area%	Coconut Oil (Brand G) /Area%	Reference Value [5] /Area%
Methyl Caproate, C6:0	1	1	1	1
Methyl Caprylate, C8:0	7	8	8	8
Methyl Caprate, C10:0	6	6	7	6
Methyl Laurate, C12:0	41	44	47	49
Methyl Myristate, C14:0	19	19	20	18
Methyl Palmitate, C16:0	11	9	9	8
Methyl Stearate, C18:0	4	4	3	3
Methyl Oleate, C18:1 (n9)	9	8	5	7
Methyl Linoleate, C18:2 (n6)	2	1	1	2

All the data in the table above are rounded off to the nearest percentage.

Results of Mustard Oil Samples (Brand H-I)

Mustard oil FAME profiles (**Fig. 7a-b**) (**Table 5**) stand out the most, as unlike the rest of the oil samples, the major peak observed is the longer chain FAME, i.e. methyl erucate [C22:1(n9)]. In the two samples tested, methyl erucate was observed to be present as the largest abundant peak at around 48% to 49%. The next highest abundant peak was observed to be methyl linoleate at around 14%. The data for the mustard oil from two different sources are very similar and are in good agreement with the reported literature values [6]. The slight variations in the results observed could be attributed to the extraction protocol used on the mustard seed, and the differences in the subspecies of the mustard plant used.

The high abundance of methyl erucate in the sample explains why mustard oil is not permitted to be used as cooking vegetable oil in certain regions like the US and EU [6][7][8][9]. In the EU and US, the limit is set at 2% [6][7][8][9]. Hence, this automation workflow potentially can be used to check for the level of erucic acid content in mustard oil for these regions. Lastly, in mustard oil, the third largest abundance is shared between methyl oleate and methyl α-linolenate, with roughly the same abundance (9% to 11%).

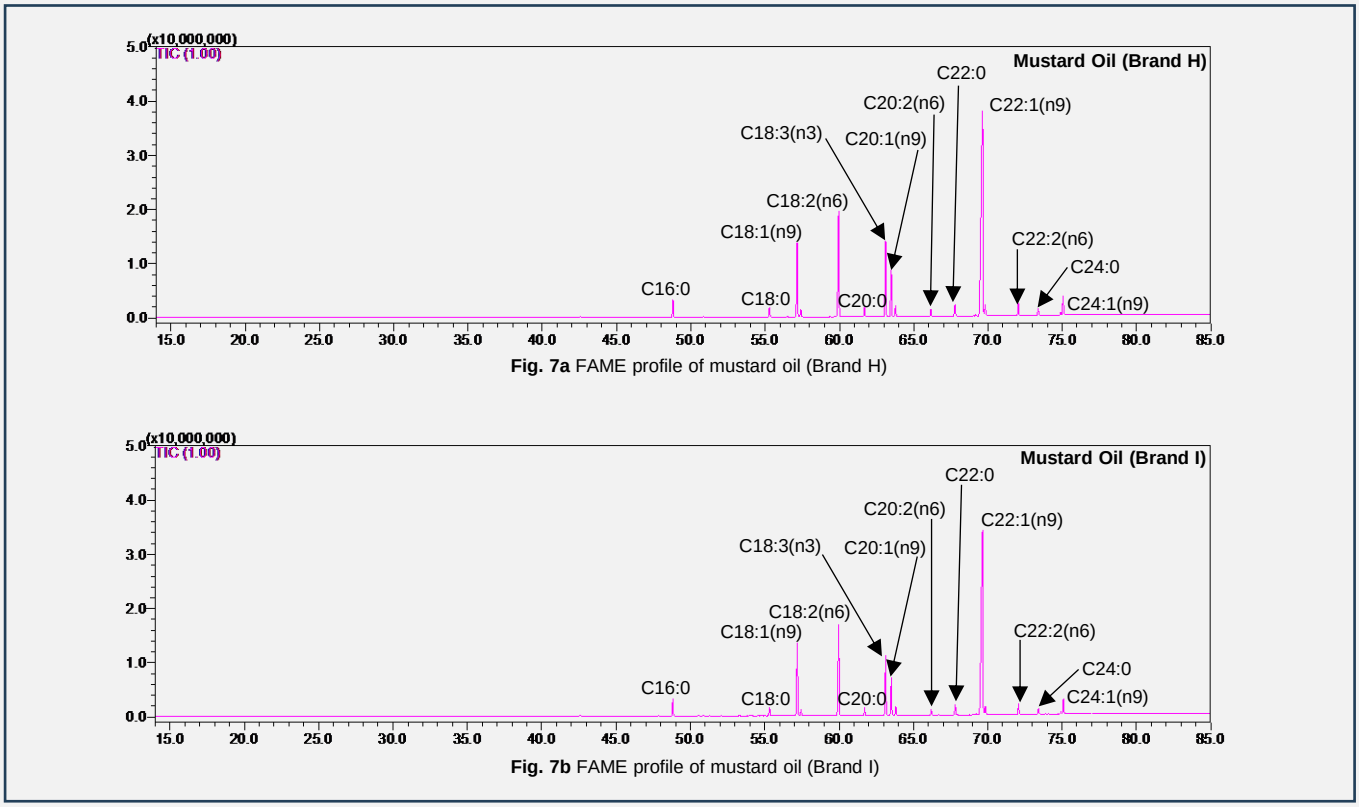


Fig. 7a FAME profile of mustard oil (Brand H)

Fig. 7b FAME profile of mustard oil (Brand I)

Table 5. Summary of the FAME Profiles of Mustard Oil Samples (TIC Area%) and its Literature Reference Values (FID Area%) [6]

Fatty Acid Methyl Ester	Mustard Oil (Brand H) /Area%	Mustard Oil (Brand I) /Area%	Reference Value [6] /Area%
Methyl Palmitate, C16:0	2	2	2
Methyl Stearate, C18:0	1	1	2
Methyl Oleate, C18:1 (n9)	9	11	16
Methyl Linoleate, C18:2 (n6)	14	14	13
Methyl Arachidate, C20:0	1	1	1
Methyl α-Linolenate, C18:3 (n3)	10	9	6
Methyl cis-11-Eicosenoate, C20:1 (n9)	6	6	7
Methyl cis-11,14-Eicosadienoate, C20:2 (n6)	1	1	0
Methyl Behenate, C22:0	2	2	1
Methyl Erucate, C22:1 (n9)	49	48	49
Methyl cis-13,16-Docosadienoate, C22:2 (n6)	1	2	0
Methyl Lignocerate, C24:0	1	1	0
Methyl Nervonate, C24:1 (n9)	2	2	2

All the data in the table above are rounded off to the nearest percentage.

Results of Olive Oil Samples (Brand J-K)

Fig. 8a-b depict the TIC FAME profiles of commercially purchased olive oil samples, and **Table 6** summarises their respective peak areas in %Area. The FAME profiles of olive oil is very similar to that of palm oil in terms of the order of abundance; however, it has a noticeably larger amount of methyl oleate detected. Methyl oleate as the highest abundant peak was observed to be around 64% to 77%.

The two chromatograms (**Fig. 8a-b**) obtained are highly identical to one another, and the results obtained (**Table 6**) agree with values found in the literature [10], however slight variations are still observed. This is likely attributed to the differences in harvesting protocol, subspecies of the olive seed used, and the extraction processes used to obtain the olive oil [10].

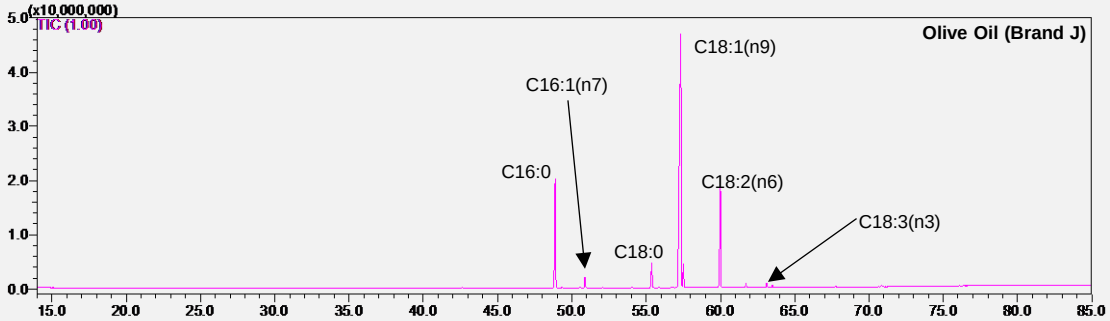


Fig. 8a FAME profile of olive oil (Brand J)

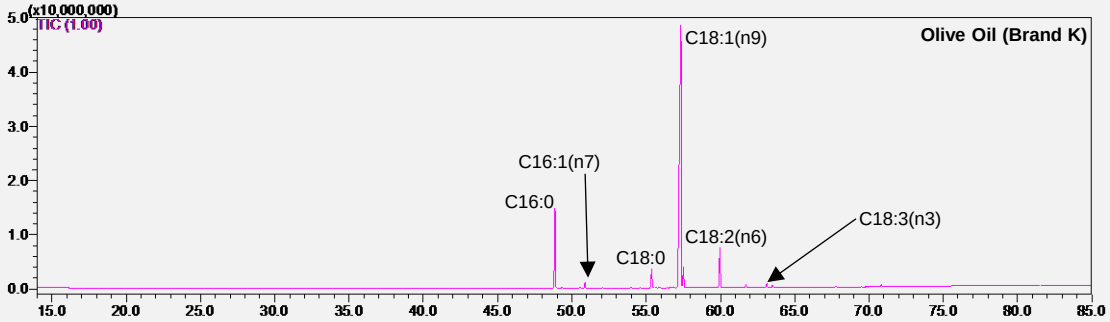


Fig. 8b FAME profile of olive oil (Brand K)

Table 6. Summary of the FAME Profiles of Olive Oil Samples (TIC Area%) and its Literature Reference Values (FID Area%) [10]

Fatty Acid Methyl Ester	Extra Virgin Olive Oil (Brand J) /Area%	Extra Virgin Olive Oil (Brand K) /Area%	Reference Value [10] /Area%
Methyl Palmitate, C16:0	16	12	14
Methyl Palmitoleate, C16:1(n7)	1	1	0
Methyl Stearate, C18:0	3	3	3
Methyl Oleate, C18:1 (n9)	64	77	63
Methyl Linoleate, C18:2 (n6)	14	6	20
Methyl α-Linolenate, C18:3 (n3)	1	1	0

All the data in the table above are rounded off to the nearest percentage.

Results of Peanut Oil Samples (Brand L)

The FAME profile for peanut oil obtained exhibits a very similar FAME profile to those of olive oils (**Fig. 8a-b** with **Fig. 9**) (**Table 6** with **Table 7**). However, it is still possible to differentiate them by looking at the lower abundant peaks. For example, there is an absence of C16:1(n7), while the longer chain fatty acids [such as methyl arachidate (C20:0), methyl behenate (C22:0), and methyl lignocerate (C24:0)] are all present in trace amounts in peanut oil. These fatty acids could potentially be used as markers to differentiate the 2 oils apart.

Peanut oil FAME profiles have about 57% methyl oleate which is slightly lower than olive oil, and a slightly higher amount of methyl linoleate at 22% as compared to olive oil. It also has a slightly lower amount of methyl palmitate at 9% with respect to olive oil. Thus, despite the similarity in the TIC profiles, it is still possible to differentiate the two oils apart. The FAME profile for the peanut oil summarised in **Table 7** is very similar to the reported value in the literature [11].

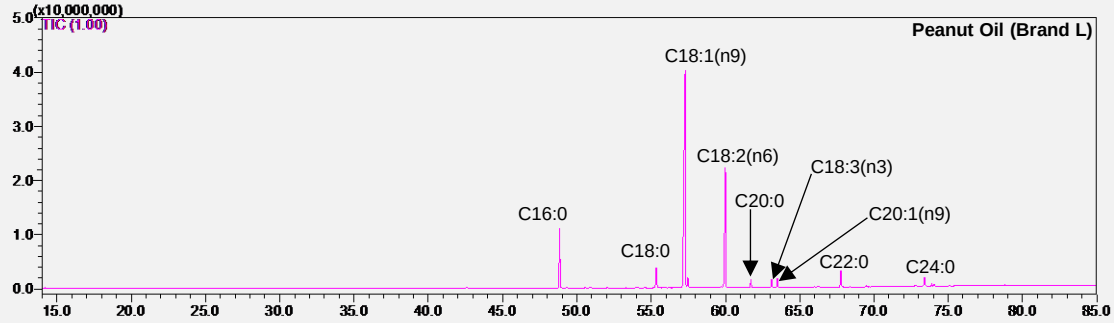


Fig. 9 FAME profile of peanut oil (Brand L)

Table 7. Summary of the FAME Profiles of Peanut Oil Samples (TIC Area%) and its Literature Reference Values (FID Area%) [11]

Fatty Acid Methyl Ester	Peanut Oil (Brand L) /Area%	Reference Value [11] /Area%
Methyl Palmitate, C16:0	9	10
Methyl Palmitoleate, C16:1(n7)	0	2
Methyl Stearate, C18:0	3	0
Methyl Oleate, C18:1 (n9)	57	52
Methyl Linoleate, C18:2 (n6)	22	30
Methyl Arachidate, C20:0	1	1
Methyl α-Linolenate, C18:3 (n3)	1	0
Methyl cis-11-Eicosenate, C20:1 (n9)	1	1
Methyl Behenate, C22:0	2	3
Methyl Lignocerate, C24:0	2	2

All the data in the table above are rounded off to the nearest percentage.

Results of Canola Oil Sample (Brand L)

Fig. 10 depicts the FAMEs profile of commercially purchased canola oil, and **Table 8** summarises the result obtained with respect to the literature reported value. Unlike the earlier data presented, canola oil was only tested with 5 mg of the sample and performed with a lower split ratio (50). This was performed to reduce the likelihood of detector saturation. The data obtained is in strong agreement with the reported literature value [12]. Slight variations were observed, this fall is within expectation which could be attributed to the sources/subspecies of the rapeseed used, and the difference in the extraction process. Excellence reproducibility was observed (**Table 8**), which clearly demonstrate the superior robustness of the proposed solution.

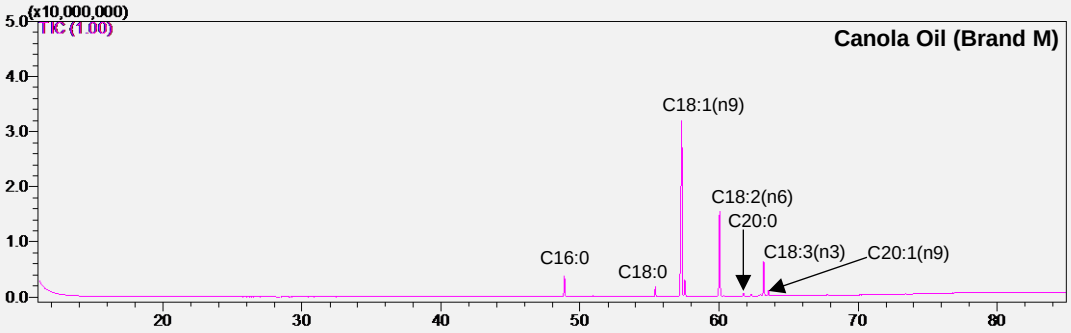


Fig. 10 FAME profile of canola oil (Brand M)

Table 8. Canola Oil FAMEs Profile (TIC Area%) Obtained vs Literature Value (FID Area%) [12]

Fatty Acid Methyl Ester	Canola Oil (Brand M) /Area%	Reference Value [12] /Area%
Methyl Palmitate, C16:0	5	3
Methyl Stearate, C18:0	2	2
Methyl Oleate, C18:1 (n9)	59	59
Methyl Linoleate, C18:2 (n6)	22	22
Methyl Arachidate, C20:0	1	1
Methyl α-Linolenate, C18:3 (n3)	8	12
Methyl cis-11-Eicosenoate, C20:1 (n9)	1	1
Methyl Erucate C22:1 (n9)	0	0

All the data in the table above are rounded off to the nearest percentage.

4.3 Repeatability and Robustness Demonstration

Mustard oil (**Fig. 7a-b**) has one of the most complex FAME profiles among all the samples analyzed. It was thus chosen for the repeatability study to demonstrate the robustness of the workflow (**Table 9**). The split ratio was increased to 200 just for this experiment, as detector saturation was observed for the peak of methyl erucate.

To demonstrate repeatability, three separate portions of mustard oil (10 mg each) were placed on the AOC-6000 Plus sample rack, and subsequently underwent the same automation workflow, followed by GCMS analysis. The results of the repeatability studies (n=3), are summarised in **Table 9**.

Comparing the results obtained (**Table 5** and **Table 9**), the data obtained are highly identical. At a higher split ratio, methyl erucate is consistently detected at 51%, slightly higher than earlier experiment when detector saturation were observed (49%) (**Table 5)**. Thus, the error that occurred due to detector saturation in Table 5 is deemed relatively insignificant.

The results in **Table 9** clearly demonstrate the strength of automation. Highly identical results are consistently obtained across all the FAME profiles peaks for the three repeated attempts. Such high reproducibility can be attributed to the nature of the automation workflow used; all the samples are freshly prepared in an identical manner and injected into the GCMS almost immediately.

Table 9. Repeatability Studies using Mustard Oil (Split Ratio 200)

Fatty Acid Methyl Ester	Mustard Oil Attempt 1 /Area%	Mustard Oil Attempt 2 /Area%	Mustard Oil Attempt 3 /Area%
Methyl Palmitate, C16:0	2	2	2
Methyl Stearate, C18:0	1	1	1
Methyl Oleate, C18:1 (n9)	9	9	9
Methyl Linoleate, C18:2 (n6)	14	14	14
Methyl Arachidate, C20:0	1	1	1
Methyl α-Linolenate, C18:3 (n3)	9	9	9
Methyl cis-11-Eicosenoate, C20:1 (n9)	6	5	5
Methyl cis-11,14-Eicosadienoate, C20:2 (n6)	1	1	1
Methyl Behenate, C22:0	2	1	2
Methyl Erucate, C22:1 (n9)	51	51	51
Methyl cis-13,16-Docosadienoate, C22:2 (n6)	2	2	1
Methyl Lignocerate, C24:0	1	1	1
Methyl Nervonate, C24:1 (n9)	2	2	2

All the data in the table above are rounded off to the nearest percentage.

5. CONCLUSION

The results presented in this Technology Brief highlight the advantages of using an automated workflow with the Shimadzu GCMS-QP2020 NX, coupled with the AOC-6000 Plus. The proposed system fully automates the sample preparation steps for fatty acid methyl ester (FAME) profiling across a diverse range of edible oil samples (**Table 3-8** and **Fig. 5-10**).

All samples were freshly prepared in an identical manner and were injected into the GCMS once ready. This approach enables users to obtain highly robust and reproducible FAME profiles. Our team used MS as detector instead of the conventional method of using FID. This is to leverage the advantage of having additional dimension of mass spectrum data for identifying unknown peaks. It has been demonstrated that the data obtained using MS is in strong agreement with the literatures that uses FID.

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