

Gas Chromatographic Analysis of Medium Chain Fatty Acids in Coconut Oil

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ABSTRACT

Analysis of medium chain of fatty acids in coconut oil becomes important due to their roles in health issues. The present analysis methods for fatty acids present in food mainly focused to the overall fatty acid concentration. The analytical method for specific medium chain fatty acids was not so much be given attention. This research was focused to the analytical methods for these particular fatty acids in coconut oil. Several analytical methods were compared including acid catalyzed, basic catalyzed and acid boron trifluoride catalyzed derivatization. The response of each fatty acid toward the derivatization methods was different. Formation of the fatty acid methyl ester from caprylic and capric was low for acid catalyzed method compared to basic catalyzed method and acid boron trifluoride catalyzed methods. This result showed that the kinetics of the esterification among the fatty acids were not the same. The analysis of all fatty acids in coconut oil is better using basic catalyzed than the other methods.

Key Words : MCFA, coconut oil, Gas Chromatography, fatty acid analysis

INTRODUCTION

Coconut oil is one of the main agriculture products in Indonesia. The product got popularity recently after the roles of the medium chain fatty acids have been revealed. Medium chain fatty acids are known having a significant role in human metabolism as antibiotics, especially as antiviral and source of fast energy without disturbing the blood sugar in the body [1].

Coconut oil contains a significant amount (up to 50 percent) of lauric acid other than caprylic and capric acid [2]. These fatty acids brought the coconut oil product such as virgin coconut oil became a very popular product. It is related to the ability of the human body to overcome the vascular diseases and diabetic diseases. Medium chain fatty acids are known able to prevent wound in the artery surface by acting as antimicrobial and to supply energy for metabolism without increase the blood sugar level in mammals [3, 4].

The methods for analyzing the medium chain fatty acids in coconut oil are not studied so well yet up to date. So far the reported methods for fatty acid analysis in coconut are mostly based on the standard methods for fatty acid analysis. On the other hand, it is known that those medium chain fatty acids are more easily to be evaporated during derivatization so it is very important to be taking care during the derivatization.

There are several methods for derivatization of fatty acids can be analyzed by gas chromatography including acid catalyzed, base catalyzed and diazomethane [5]. Acid catalyzed using boron trifluoride is widely used in fatty acid derivatization and had adopted

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by International Union Pure and Applied Chemistry [5, 7, 8]. This method is claimed to be fast and efficiently and recommended by American Oil Chemists Society [9]. Nevertheless, Christie [5] pointed out that there are several drawbacks of this boron trifluoride method including the reagent were not stable for long time storage and some methoxy artifact formation. Other acid catalyzed methods using hydrochloric or sulfuric acids are able to convert free fatty acid as well as esterified fatty acids. The drawback of this method was taking longer derivatization time. The basic derivatization method is able to derive in short time but not able to convert free fatty acids into methyl ester. The diazomethane method is suffer from the reagent is very unstable for storage.

Gas chromatographic analysis of coconut oil has been reported for various purposes using various derivatization methods including base catalyzation [10], acid catalyzation [11] and acid trifluoride catalyzation [12], but no one give special attention on the derivatization methods. There is no report on the medium fatty acid derivatization including their kinetic of reaction and the efficiency compared to derivatization of other longer fatty acids. The main purposes of this work are to study the best method for determination of medium chain of fatty acids in coconut oil.

EXPERIMENT

Materials

The coconut oils were purchased from the local markets. Sodium hydroxide, sulfuric acid, methanol, hexane, sodium chloride (NaCl), hydrochloric acid (HCl) and acetic acid, and sodium sulfate were purchased from BDH as analytical grade. The boron trifluoride methanol solution (15%) and standard fame mix C₈-C₂₄ were purchased from Sigma/Supelco.

Derivatization Methods

Acid catalyzed derivatization method [5]

50 mg of an aliquot sample was weighed in each of two screwed cap test tubes (10 mL) and was added 400 µL of 1% methanolic H₂SO₄. The mixtures were heated at 50 °C for 2 and 4 hours respectively. After cooled, 1 mL of 15% NaCl was added, and then 1 mL hexane was added successively. The mixtures were vortex then settled for couple of minutes. The mixture formed two layers. The top layer was collected for further gas chromatographic analysis.

Base catalyzed derivatization method [5]

50 mg of an aliquot sample was weighed in a screwed cap test tubes (10 mL) and was added 400 uL of 0.5 M methanolic NaOH. The mixture then heated at 50 °C for 20 seconds. Then the mixture was added 1 mL of hexane and mixed utilizing vortex for 30 seconds. After settled, two layers will be formed. The top layer was transferred into a clean tube, was added 400 mL of 2 N methanolic HCl, and then mixed utilizing vortex. After it was settled for couple minutes, the top layer was removed and for further analyzed by gas chromatographic.

Boron trifluoride catalyzed derivatization method [13]

50 mg of an aliquot sample was weighed in a screwed cap test tube (10 mL) and added 1 mL of hexane then mixed utilizing vortex. After that 1.5 mL of NaOH was added and heated at 80 °C for 5 minutes. After cooled, 1 ml of 12% BF₃ in methanol was added and the mixture was heated at 80 °C for 25 minutes. After cooled, added 1.5 mL of hexane and 3 mL of saturated NaCl respectively. The top layer was removed into a clean test tube and added some Na₂SO₄ filtered and ready to be analyzed by gas chromatographic.

Chromatographic Analysis

Chromatographic analysis was performed by Shimadzu Gas Chromatography (GC-2014) with Flame Ionization Detector (FID) and Capillary Fused Silica Column (Rtx – Wax) 30 meter length, 0.25 mm inner diameter and 0.25 μm film thickness. The column temperature was set as follow 120 $^{\circ}\text{C}$ isothermal for 7 minutes, gradually increment 10 $^{\circ}\text{C}$ per minute for 26 minutes up to 240 $^{\circ}\text{C}$ then isothermal for 12 minutes. The injection room was heated at 240 $^{\circ}\text{C}$. Injection mode was set as split mode with 1/10 and the pressure of helium gas was set at 75 kPa. One milliliter of samples was injected into the injection port.

Standard Solution Preparation

The standard solution from the supplier (Sigma) was taken from the ampule and added into 1 mL of hexane. The solution concentration of 100 mg/mL was stored for further gas chromatographic analysis. For the purpose of external standard the solution was diluted to 50, 20, 10, 5, 2 mg/mL respectively.

RESULTS AND DISCUSSION

Optimization of gas chromatographic equipment

The temperature program for gas chromatographic equipment was started by 70 $^{\circ}\text{C}$ and increased to 220 $^{\circ}\text{C}$, but it was found that the analytical time took so long which was more than 60 minutes. Therefore, the initial temperature was set at 120 $^{\circ}\text{C}$ followed by increased gradually 10 $^{\circ}\text{C}$ per minute up to 240 $^{\circ}\text{C}$ then hold for another 12 minutes. At this column condition give a reasonable chromatogram as shown in **Figure 1**. The medium chain methyl fatty acids eluted in a reasonable time and very well resolved. The fatty acid of C-24 did not show due to its elution time was 42.5 minutes.

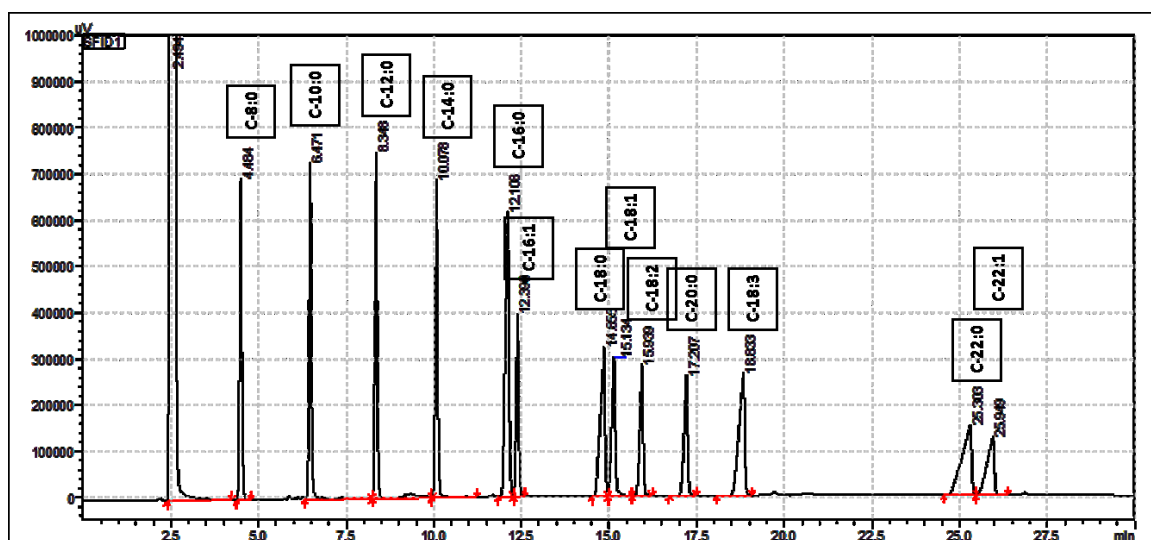


Figure 1. Chromatogram of fatty acid methyl ester standard

Analysis of coconut fatty acids

Using the developed gas chromatographic program, the fatty acid methyl ester from coconut oil was analyzed. The result can be seen in **Figure 2**. The total elution time was about 20 minutes. The fatty acid methyl esters were separated very well.

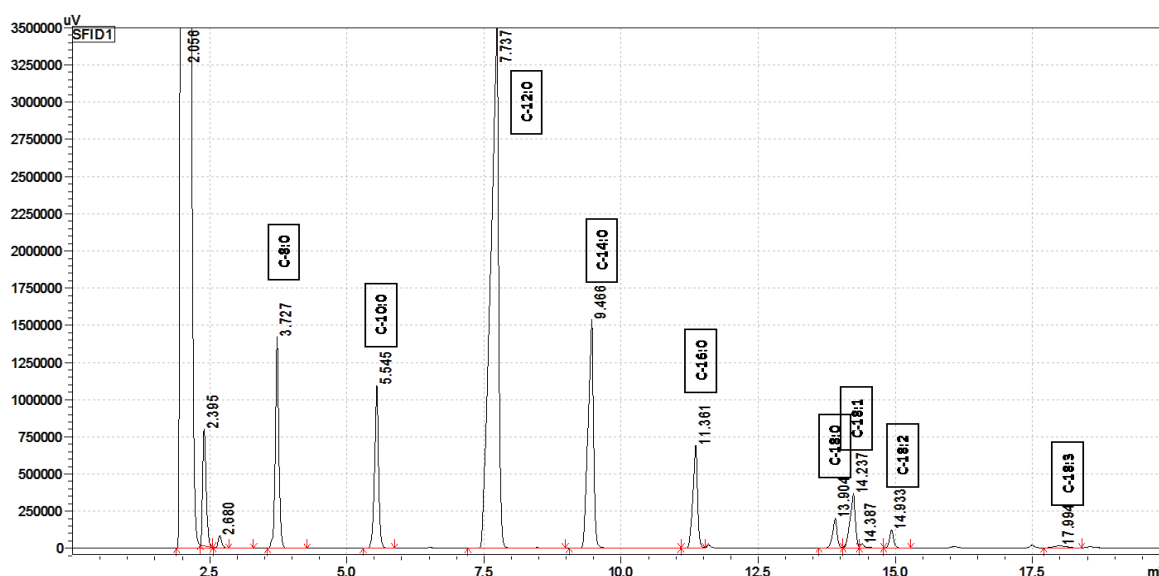


Figure 2. Chromatogram of Fatty Acid Methyl Ester from Coconut Oil

Efficiency of derivatization

The efficiency of derivatization methods can be seen in **Table 1**. Acid catalyzed transesterification produced the lowest transesterification, 15.1 and 21.4 mg/mL or 30 and 43 %, respectively for 2 and 4 hour derivatization time. According to Christie [5] the acid catalyzed transesterification take longer time than base catalyzed transesterification. Base catalyzed derivatization give the highest conversion efficiency (112 %) and has a shortest derivatization time (20 seconds). The conversion is higher than 100 percent might due to the solvent evaporation during solution transferring. These finding agree with the previous reports that base catalyzation derivatization has shorter derivatization time and higher derivatization efficiency [14, 15, 16].

Table 1. Absolute concentration and conversion efficiency of fatty acids after derivatization methods.

Fatty Acids	Absolute Concentration (mg/ml) / Conversion Efficiency (%)			
	Base Transesterification	BF ₃ Transesterification	Acid Transesterification	
			2 hours	4 hours
C8:0 (Caprylic)	6.1 (12)	6.2 (12)	2.0 (4.0)	3.1 (6.2)
C10:0 (Capric)	3.8 (7.6)	3.9 (7.8)	1.2 (2.4)	1.7 (3.4)
C12:0 (Lauric)	25.8 (52)	24.2 (48)	7.1 (14)	10.3 (21)
C14:0 (Myristic)	9.8 (19.6)	7.7 (15)	2.4 (4.8)	3.5 (7.0)
C16:0 (Palmitic)	4.5 (9.0)	2.8 (5.6)	1.1 (2.2)	1.4 (2.8)
C18:0 (Stearic)	1.4 (2.8)	0.8 (1.6)	0.4 (0.8)	0.4 (0.8)
C18:1 (Oleic)	3.9 (7.8)	1.6 (3.2)	0.8 (1.6)	0.8 (1.6)
C18:2 (Linoleic)	0.7 (1.4)	1.0 ((2.0)	0.2 (0.4)	0.2 (0.4)
Total	56.0 (112)	48.1 (96)	15.1 (30)	21.4 (43)

The medium chain fatty acid methyl ester especially for caprylic and capric were low for base catalyzation compared to the BF₃ transesterification. These findings indicate that the lower medium chain fatty acids were more readily converted by BF₃ catalyzed derivatization than by base catalyzed derivatization.

Based on these findings it showed that for overall methods the base catalyzed derivatization is the best method for two reasons that are the highest conversion efficiency and the shortest derivatization time but for lower medium chain fatty acid, the BF_3 catalyzed method is better conversion.

CONCLUSION

The base catalyzation method is the best for fatty acid analysis in coconut oil and the BF_3 catalyzation method is the best for lower medium chain fatty acid analysis.

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