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Profile of Monoglyceride and Diglyceride Compounds of the Ethanolysis Products from Palm Kernel Oil (PKO)

Murhadi, S Hidayati, R Sugiharto

Department of Agricultural Product Technology, Faculty of Agriculture, University of Lampung, Jalan Soemantri Brojonegoro No. 1 Bandar Lampung, Lampung Province, Indonesia 35145

Corresponding main author: murhadiburcik@gmail.com

Abstract. Ethanolysis of PKO has been done by the addition of ethoxide ion solution {ethanol 90% containing 1% (w/w PKO) of NaOH} to 100 g PKO at the ratio of 1.0; 1.2; or 1.4 (w/w), each with the addition of glycerol 10% (w/w PKO) at 55-60°C, stirred at 1000 rpm for 3, 6, 9, or 12 minutes. Careful observation is done on the yield and monoglyceride, diglycerides, and triglycerides distribution patterns in the PKO ethanolysis products. The average yield of all treatments resulted in a value of 44.91% (\pm 4.47%; w/w). The yield of ethanolysis of PKO with the ratio of 1.4 (w/w) is better than with the ratio of 1.0 (w/w) and 1.2 (w/w), while 6 minutes shown a better result for the PKO ethanolysis time reaction compared to 3, 9, or 12 minutes. The average of glycerides, esters, monoglycerides, internal standard, diglycerides, and triglycerides in overall treatments, shows 6.09, 55.02, 7.07, 2.55, 23.54, and 5.73%, respectively. Group of monoglyceride (MG) and diglyceride (DG) compounds in PKO ethanolysis products, both obtained on average with the best treatment at ethanolysis time for 3 minutes for a ratio of 1.0 (w/w), each at 7.74% (w/w) and 30.60% (w/w).

Keywords: monoglycerides, diglycerides, ethanolysis, PKO.

1. Introduction

The growth of the planting area of oil palm plantations (*Elaeis guineensis* Jacq) in Indonesia tends to increase with an average in 2004 - 2014 of 7.67%, while oil palm production also increases by an average of 11.09% per year. Based on the Statistics Book of Oil Palm Plantations 2015, Direktorat Jenderal Perkebunan, in 2016 (predictive figures) the area of oil palm reached 8.77 million Ha with a production of 33.50 million tons of CPO and productivity reaching 3.79 tons / Ha [1]. The tendency of increasing production from the industries of crude palm oil (CPO, reddish orange) in Indonesia, the production of palm kernel oil (PKO, yellow) produced from the palm kernel seeds will also continue to increase from year to year.

The oil content in palm kernel seeds is around 50 percent [2]. The types of fatty acids in PKO consist of at least 9 types, which are divided into 7 types of saturated fatty acids which are dominated by lauric acid (C12:0; 49.39%) and myristic acid (C14:0; 15.35%), 2 types of unsaturated fatty acids dominated by oleic acid (C18:1; 15.35%) and linoleic acid (C18:2; 3.10%), and the rest (0.07%) in the form of minor fatty acids not yet identified [3]. Lauric acid is the main ingredient in coconut oil and palm kernel oil which can form monolaurin (monoglyceride) in the body which has the potential as a high antimicrobial compound. In vivo experiments in mice showed that oregano and monolaurin oils, both individually and in combination, were as effective as some antibiotics, so they could potentially be antimicrobial agents [4]. Some research results show that MG products in the form of monolaurin and monomiristine have been shown to have anti-bacterial activity (Gram + and Gram -), anti-yeast activity, and anti-mold activity with broad-spectrum [5, 6, 7, 8], even proven as anti-tumor cells and HIV-1 [9]. Monoglycerides from certain types of fatty acids obtained from several plant sources, has been shown to have antimicrobial activity with a broad spectrum, especially for monolaurin, monocaprin, and monomeristin types [5].



Research for the synthesis and production of monoglyceride (MG) and diglyceride (DG) emulsifier products and or a mixture of both from various sources of natural ingredients has been carried out since ancient times, especially using high temperatures (chemical glycerolysis). Results of research by [10, 11, 12, 13, 14, 15] has proven that PKO and / or mixtures with CPO crude palm oil have the potential to be further processed into derivative products that have functions as antimicrobial compounds that can be used as food preservatives.

Ethanolysis reaction using 96% ethoxide ion solution {containing 1% NaOH (w / w PKO)} to PKO at room temperature (28-30°C) with the addition of 10% (w/w PKO) glycerol, stirred at 1000 rpm for 3 minutes, resulting in 18% (w/w) of crude PKO ethanolysis product which is able to preserve fresh coconut milk and fresh cow's milk stored at 25-30°C (open packaging) for more than 4 days (control after 1 day storage very foul) [16]. As a result of the temporary identification, the product contains a collection of functional fatty acids (8:0, 10:0, 12:0 and 14:0) reaching 41%, especially 12:0 reaching 26%. The rest are the remaining glycerol which has not reacted 40%, methyl esters 9%, ethyl esters 2%, DG 1%, other fatty acids 4% and other components 3% [16]. Other tests showed that ethanolysis reaction time for 1, 3, 5, 7, 9, 11 and 13 minutes resulted in product yield, emulsion stability, and power of coconut milk preservation which was not significantly different if carried out at room temperature (25-30°C) [16]. Therefore, the next study was directed to perform the reaction of PKO ethanolysis at a higher temperature (55-60°C) at different times reaction and the ratio of ethoxide ion to PKO solutions, so that the yield of PKO ethanolysis products could be maximal. The composition and distribution patterns of MG and DG compounds in PKO ethanolysis products are unknown. This research was aimed to identification of profile of monoglyceride and diglyceride compounds of the ethanolysis products from Palm Kernel Oil (PKO).

2. Materials and Equipment

The main ingredient is fresh palm kernel oil (PKO) which is obtained directly from the PTPN VII palm kernel oil processing industry in the Bekeri, Central Lampung, Lampung Province. Chemicals include ethanol absolute p.a., technical ethanol, NaOH, 35% HCl, glycerol, and a number of chemicals for analysis of monoglycerides, diglycerides and other compounds with gas chromatography (GC). Equipment used to include a set of 3-neck glass reflux flasks (1 L) for ethanolysis reaction, hot plate magnetic stirrer, separating funnel, rotary evaporator, GC, and supporting devices.

3. Making Ethoxide Ion Solution

Ethoxide ion solution was made by mixing 90% ethanol with 1% (w/w PKO) NaOH. One of the 90% ethanol ratios that have contained 1% NaOH against PKO used is 1.0 (w/w). What is meant by 1% (w/w PKO) NaOH is the weight of NaOH 1% of the weight of PKO. So in one experimental unit that uses 80.00 g PKO, it takes 0.80 g NaOH in 80 g of 1% NaOH ethanol solution. Ethanol used is ethanol absolute (100%), so it needs mixing of distilled water around $10 \times 100/90 = 11.11\%$ of 80 g of 100% ethanol, equivalent to 8.88 g of distilled water. As much as 0.80 g NaOH was dissolved in 8.88 g of distilled water until it dissolved completely (heat will arise) and then mixed into absolute ethanol until the weight of 80 g of the mixture was mixed, so that ethoxide ion solution was produced of 90% ethanol containing 1% NaOH (w/w PKO). Furthermore, with the same calculation, it is carried out for the ratio 1.2 and 1.4 (w/w).

4. Production of PKO Ethanolysis Products

The equipment for the reaction of PKO ethanolysis using a 3-neck glass reflux flask (Figure 1) was carried out at a temperature of 55-60°C with a hot plate magnetic stirrer, stirred at 1000 rpm. The treatments (3 replications) consist of: (1) stirring time during PKO ethanolysis, consisting of 3, 6, 9 and 12 minutes; and (2) ratio of ethoxide ion solution to fresh PKO, consisting of 1.0; 1.2; and 1.4 (w/w). PKO ethanolysis reaction was carried out following the methods from Murhadi and Hidayati [16] and Murhadi [3] with modifications.

A number (g, according to the treatment ratio) of ethoxide ion solution was added 80 g PKO in a 3-neck glass reflux flask, then placed on a hot plate magnetic stirrer with stirring speed 1000 rpm for several minutes (according to the ethanolysis time treatments) at the reaction temperature of 55-60°C. The reaction was stopped by dropping 20 drops of HCL solution (35%). The reaction product mixture was put into a separator flask for 30 minutes, so that there is a clear separation between two layers. The top layer (pale yellow ethanolysis product) is separated from the lower layer (remaining PKO etc, bright yellow).

5. Observation

Observations included the yield of PKO ethanolysis products and analysis of monoglycerides and diglycerides profiles in PKO ethanolysis products (using Gas Chromatography / GC). The crude ethanolysis product yield from PKO was calculated by dividing the weight (g) of crude ethanolysis products from PKO by the weight (g) of the PKO which was reacted, then multiplied by 100%.

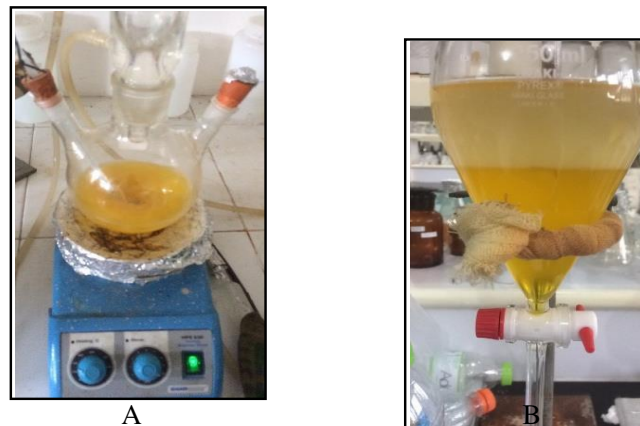


Figure 1. A 3-neck glass reflux flask (A) and separation of PKO ethanolsis products using separator flasks (B)

6. Results and Discussion

The yield of PKO Ethanolsis Products

Graph of yield of PKO ethanolsis products is presented in Figure 2. The average yield of all the treatments, both of the reaction time and the ratio of ethoxide ion solution to PKO (w/w) in PKO ethanolsis reactions resulted in a value of 44.91% ($\pm 4.47\%$; w/w). The lowest yield of PKO ethanolsis product was 27.45 (± 1.42 ; w/w) of the ethanolsis reaction time for 3 minutes and the ratio of 1.0 (w/w), while the highest ethanolsis PKO yield was 67.79 (± 2.82) of the ethanolsis reaction time for 6 minutes and the ratio of 1.4 (w/w).

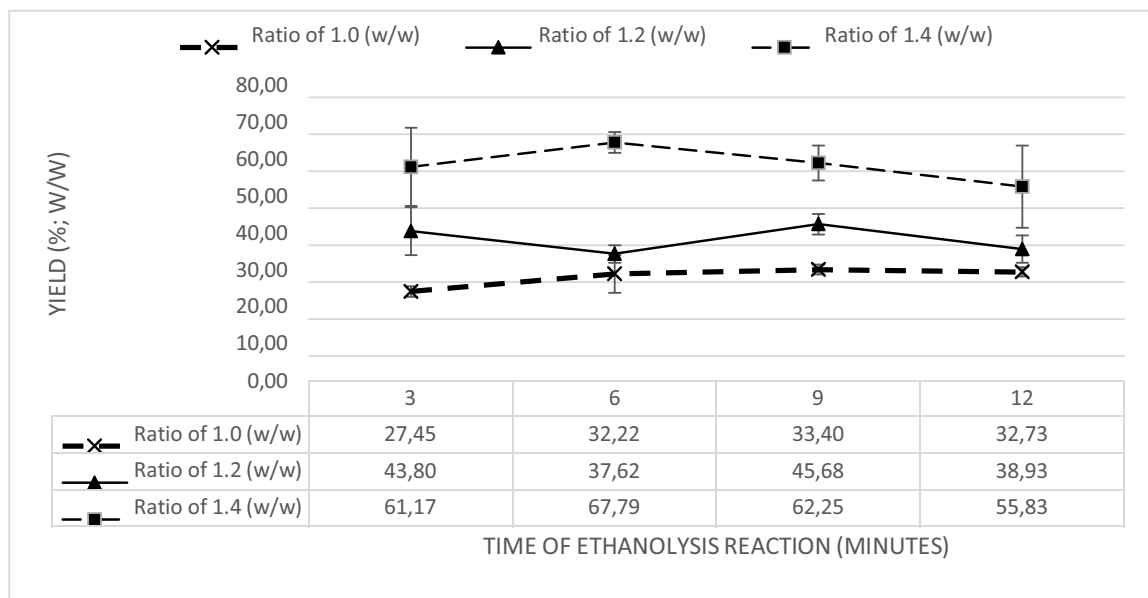


Figure 2. Effect of time of ethanolsis reaction (minutes) and ratio of ethoxide ion solution to PKO (w/w) to the yield of PKO ethanolsis products.

Figure 2 shows that the treatment with ratio of ethoxide ion solution to PKO of 1.4 (w/w) is better than other ratios of 1.0 and 1.2 (w/w), while the treatment with time of PKO ethanolsis reaction for 6 minutes

is relatively better than the reaction time of 3, 9 and 12 minutes. On average with the higher ratio of ethoxide ion solution to PKO (w/w) up to the ratio of 1.4 (w/w) further increases the yield of PKO ethanolysis products, presumably because the increase presence of ethoxide ion solution which increases the ethanolysis reaction to produce PKO ethanolysis products. While the ethanolysis time treatment which is higher than 6 minutes is relatively not increasing the yield of PKO ethanolysis products. Presumably there is a back and forth reaction between the reacting substrate (PKO) and ethanolysis products produced, so that there is a certain optimal time in PKO ethanolysis process.

The alcoholysis reaction or the common transesterification reaction applied to triglycerides is the reaction between triglycerides and alcohol (with methanol as methanolysis or with ethanol as ethanolysis reactions) and generally using alkaline catalysts (Figure 3). Without a catalyst, the reaction actually takes place very slowly [17].

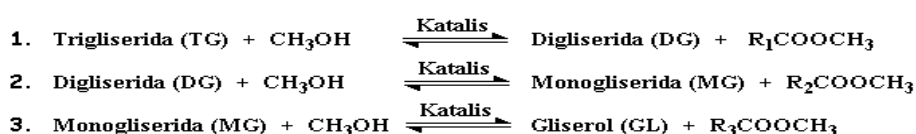


Figure 3. The methanolysis reaction takes place in three stages [17].

The most popular base catalysts for the transesterification reaction are sodium hydroxide, potassium hydroxide, sodium methylate (methoxide), and potassium methylate. The true catalyst for the actual reaction is the methylate ion (methoxide) which, if the catalyst added is hydroxide [17]. If ethanol and NaOH are used, ethylate (ethoxide) ions will be produced which are very important in the reaction of PKO ethanolysis. Furthermore, ethanolysis reaction on vegetable oil, especially in TG through three stages of reaction, namely: (1) triglycerides react with ethanol in alkaline conditions to produce DG and ethyl ester first from the position of fatty acids to 1 / sn-1, (2) diglycerides then react with excess ethanol residues in alkaline conditions produce MG and second ethyl esters from the position of the third fatty acid / sn-3, and (3) if the reaction continues, MG will react with excess ethanol remaining producing the third glycerol and ethyl esters [18, 19].

7. Profile of Monoglyceride and Diglyceride Compounds in PKO Ethanolysis Products

One of the chromatograms of Gas Chromatography (GC) results of the identification of a group of chemical compounds (MG, DG, TG, esters, other substances) in PKO ethanolysis products carried out in the Oil Palm Research Center (PPKS) Medan, North Sumatra, is presented in Figure 4. Furthermore, the data recapitulation of the group of chemical compounds in the PKO ethanolysis products, presented in Table 1.

Based on the data in Table 1, the average content of glycerides, esters, monoglycerides, internal standard, diglycerides and triglycerides in PKO ethanolysis products for all treatments (2 replications) are 6.09; 55.02; 7.07; 2.55; 23.54; and 5.73 percent, respectively. The results obtained by an average of 7.07 percent of monoglycerides content, with the highest value (7.97 percent) at 9 minutes ethanolysis time treatment for the ratio of ethoxide ion solution to PKO of 1.0 (w/w), also followed by the treatment of ethanolysis time for 3 minutes for the same ratio of 1.0 (w/w) with a value of 7.74 percent, while the lowest value (6.33 percent) in the ethanolysis time treatment during 6 minutes to ratio of 1.2 (w/w). Furthermore, for the average content of diglycerides reached 23.54 percent, with the highest value (30.60 percent) ethanolysis time treatment for 3 minutes for the ratio of 1.0 (w/w), while the lowest value (19.62 percent) in the treatment of ethanolysis time for 6 minutes for the ratio of 1.4 (w/w). Based on some results of previous studies, it is known that the presence of monoglyceride content formed mainly from lauric acid (C12:0) and or myristic acid (C14:0) esters is the main antimicrobial component in PKO ethanolysis products [5, 6, 7, 8, 4].

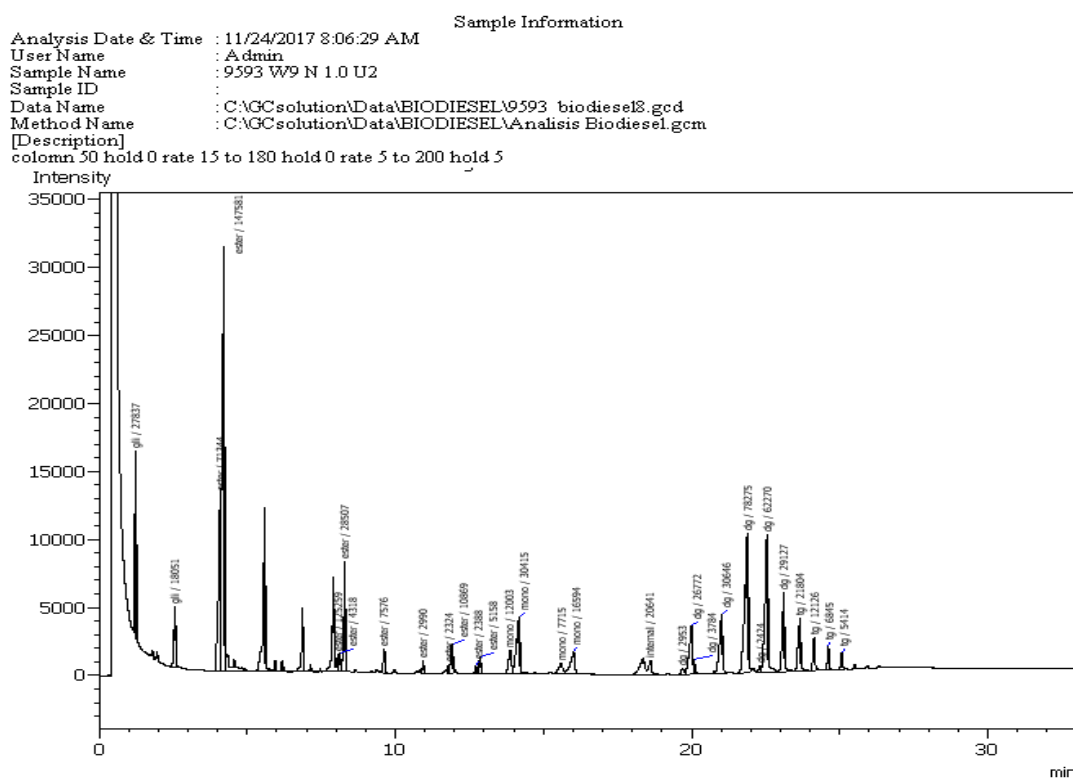


Figure 4. One of the GC chromatograms showing the profile and distributions of MG, DG, TG, esters, glycerol in PKO ethanolysis products.

Table 1. Recapitulation of data on groups of chemical compounds in PKO ethanolysis products

No.	Code	Compounds	Retention Time (RT; minutes)	Number of Compounds	Total (%)	Total MG (%)	Total DG (%)
1	W3N1-0	Glycerol	1,280 - 2,652	3 - 4	5,65	7,74	30,6
		Esters	3,759 - 12,949	6 - 20	46,29		
		Monoglycerides (MG)	13,252 - 16,398	4	7,74		
		Internal	18,609 - 17,057	1	2,68		
		Diglycerides (DG)	19,188 - 23,227	5 - 6	30,6		
		Triglycerides (TG)	23,776 - 25,608	4 - 5	7,04		
2	W3N1-2	Glycerol	1,063 - 2,682	2 - 4	5,46	6,9	24
		Esters	3,754 - 13,020	9 - 17	55,88		
		Monoglycerides (MG)	13,229 - 16,718	4 - 6	6,9		
		Internal	17,054 - 18,976	1	2,25		
		Diglycerides (DG)	20,266 - 23,307	7 - 8	24		
		Triglycerides (TG)	23,148 - 25,009	3 - 5	5,52		
3	W3N1-4	Glycerol	1,059 - 2,633	2 - 4	5,47	6,45	21,19
		Esters	3,748 - 12,409	7 - 18	56,83		
		Monoglycerides (MG)	13,220 - 16,258	4	6,45		
		Internal	17,042 - 18,396	1	2,5		
		Diglycerides (DG)	18,779 - 23,200	5 - 7	21,19		
		Triglycerides (TG)	23,747 - 25,428	4 - 6	7,56		

		Glycerol	1,053 - 2,629	3 - 4	7,03		
		Esters	3,868 - 12,934	9 - 16	50,59		
4	W6N1-0	Monoglycerides (MG)	13,216 - 16,203	4	6,79	6,79	26,36
		Internal	17,001 - 18,376	1 - 2	2,75		
		Diglycerides (DG)	18,750 - 23,189	6 - 7	26,36		
		Triglycerides (TG)	23,730 - 25,584	4 - 5	6,49		
		Glycerol	1,056 - 2,643	4 - 5	7,29		
		Esters	3,762 - 12,936	11 - 22	58,8		
5	W6N1-2	Monoglycerides (MG)	13,203 - 16,218	4	6,33	6,33	21,12
		Internal	17,001 - 18,763	1	2,23		
		Diglycerides (DG)	19,067 - 23,174	7	21,12		
		Triglycerides (TG)	23,615 - 25,143	3 - 4	4,23		
		Glycerol	1,063 - 2,574	3 - 5	5,95		
		Esters	3,774 - 12,856	11 - 26	58,5		
6	W6N1-4	Monoglycerides (MG)	13,218 - 16,043	4	6,76	6,76	19,62
		Internal	17,001 - 18,625	1	2,5		
		Diglycerides (DG)	18,739 - 23,114	7	19,62		
		Triglycerides (TG)	23,142 - 25,518	5 - 6	6,68		
		Glycerol	1,054 - 2,570	2 - 4	5,92		
		Esters	3,897 - 12,855	11 - 17	54,16		
7	W9N1-0	Monoglycerides (MG)	13,223 - 16,018	4	7,97	7,97	25,19
		Internal	17,015 - 18,609	1	2,82		
		Diglycerides (DG)	18,764 - 23,125	8	25,19		
		Triglycerides (TG)	23,625 - 25,070	3 - 4	3,94		
		Glycerol	1,051 - 2,566	2 - 4	5,44		
		Esters	3,867 - 12,840	11 - 22	52,59		
8	W9N1-2	Monoglycerides (MG)	13,214 - 15,990	4	7,08	7,08	24,94
		Internal	17,006 - 18,601	1	2,62		
		Diglycerides (DG)	18,764 - 23,107	6 - 7	24,94		
		Triglycerides (TG)	23,148 - 25,502	5 - 6	7,33		
		Glycerol	1,041 - 2,569	2 - 4	5,38		
		Esters	3,852 - 12,849	13 - 16	57,73		
9	W9N1-4	Monoglycerides (MG)	13,206 - 16,030	4	7,31	7,31	20,75
		Internal	17,001 - 18,611	1	2,7		
		Diglycerides (DG)	18,741 - 23,101	7 - 8	20,75		
		Triglycerides (TG)	23,130 - 25,501	5	6,13		
		Glycerol	1,053 - 2,558	2 - 4	6,24		
		Esters	3,747 - 12,845	9 - 21	51,29		
10	W12N1-0	Monoglycerides (MG)	13,203 - 15,999	4	7,41	7,41	26,75
		Internal	17,071 - 18,601	1	2,76		
		Diglycerides (DG)	18,735 - 23,101	6 - 7	26,75		
		Triglycerides (TG)	23,126 - 25,058	4	5,56		
		Glycerol	1,050 - 2,557	2 - 5	7,18		

		Glycerol						
		Esters	3,749 - 12,827	11 - 18	58,5			
11	W12N1-2	Monoglycerides (MG)	13,213 - 15,942	4	7,1	7,1	21,22	
		Internal	17,076 - 18,606	2	2,45			
		Diglycerides (DG)	18,732 - 23,124	7 - 8	21,22			
		Triglycerides (TG)	23,626 - 25,048	3 - 4	3,54			
		Glycerol	1,046 - 2,546	3 - 4	6,06			
		Esters	3,736 - 12,798	20 - 24	59,08			
12	W12N1-4	Monoglycerides (MG)	13,167 - 15,877	4	7	7	20,73	
		Internal	17,076 - 18,606	1 - 2	2,37			
		Diglycerides (DG)	18,665 - 23,093	7 - 8	20,73			
		Triglycerides (TG)	23,591 - 25,451	4 - 5	4,75			
						Average	7,07	23,5

Description of CODE:

- 1 = W3N1,0 : time of reaction = 3 minutes and ratio of ethoxide ion solution to PKO = 1.0 (w/w)
- 2 = W6N1,0 : time of reaction = 6 minutes and ratio of ethoxide ion solution to PKO = 1.0 (w/w)
- 3 = W9N1,0 : time of reaction = 9 minutes and ratio of ethoxide ion solution to PKO = 1.0 (w/w)
- 4 = W12N1,0 : time of reaction = 12 minutes and ratio of ethoxide ion solution to PKO = 1.0 (w/w)
- 5 = W3N1,2 : time of reaction = 3 minutes and ratio of ethoxide ion solution to PKO = 1.2 (w/w)
- 6 = W6N1,2 : time of reaction = 6 minutes and ratio of ethoxide ion solution to PKO = 1.2 (w/w)
- 7 = W9N1,2 : time of reaction = 9 minutes and ratio of ethoxide ion solution to PKO = 1.2 (w/w)
- 8 = W12N1,2 : time of reaction = 12 minutes and ratio of ethoxide ion solution to PKO = 1.2 (w/w)
- 9 = W3N1,4 : time of reaction = 3 minutes and ratio of ethoxide ion solution to PKO = 1.4 (w/w)
- 10 = W6N1,4 : time of reaction = 6 minutes and ratio of ethoxide ion solution to PKO = 1.4 (w/w)
- 11 = W9N1,4 : time of reaction = 9 minutes and ratio of ethoxide ion solution to PKO = 1.4 (w/w)
- 12 = W12N1,4 : time of reaction = 12 minutes and ratio of ethoxide ion solution to PKO = 1.4 (w/w)

The mechanism of the formation of the fatty acid methyl ester product in each of its catalytic cycles is as follows (a similar mechanism takes place in the conversion of diglycerides to monoglycerides and monoglycerides to glycerol). With a base catalyst, the methanolysis reaction can take place quickly at relatively low temperatures (room temperature to the normal boiling point of methanol, which is 65°C). Because of this, most industrial / commercial processes operate in this temperature range and atmospheric pressure; The added catalyst is usually as much as 0.5–1.5 percent of the weight of the oil treated [17].

8. Conclusion

Based on of the yield of PKO ethanolysis product, the treatment of ratio of ethoxide ion solution to PKO of 1.4 (w/w) is better than the treatment with ratio of 1.0 and 1.2 (w/w), while the treatment of PKO ethanolysis reaction time for 6 minutes is relatively better than the reaction time of 3, 9 and 12 minutes. The average yield of all treatments both the treatment of ethanolysis reaction time and the treatment of ratio of ethoxide ion solution to PKO resulted in a value of 44.91% ($\pm 4.47\%$; b / b). The average number of glycerides, esters, monoglycerides, internal standard, diglycerides, and triglycerides in all treatments, both the treatment of ethanolysis reaction time (3, 6, 9 and 12 minutes) and the treatment of ratio of 1.0, 1.2 and 1.4 (w/w), yielding values: 6.09; 55.02; 7.07; 2.55; 23.54; and 5.73 percent, respectively. The average of 7.07 percent of monoglycerides content, with the highest value (7.97 percent) at 9 minutes ethanolysis time treatment for the ratio of ethoxide ion solution to PKO of 1.0 (w/w), also followed by the treatment of ethanolysis time for 3 minutes for the same ratio of 1.0 (w/w) with a value of 7.74 percent. Furthermore, for the average content of diglycerides reached 23.54 percent, with the highest value (30.60 percent) ethanolysis time treatment for 3 minutes for the ratio of 1.0 (w/w).

9. Acknowledgment

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